Transfusion-transmitted CMV in the era of leukoreduction: Does CMV-U = CMV IgG Neg?

Oksana Prokopchuk-Gauk, MD, FRCPC (Hematology)  
Interim Head, Division of Transfusion Medicine  
Royal University Hospital, Saskatoon Health Region  
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Objectives

- Outline CMV epidemiology and clinical definitions
- Describe CMV reduction strategies and donor testing at Canadian Blood Services
- Summarize the literature demonstrating that CMV-U (CMV safe) and CMV seronegative components are equivalent in safety in BMT patients
- Review the risk of transfusion-transmitted CMV in the era of pre-storage leukoreduction
Outline CMV epidemiology and clinical definitions
CMV: The Virion

- Ubiquitous opportunistic pathogen
- ß-herpesvirus family
- 120-200 nm particles; 235 kb dsDNA
- Causes enlargement of infected cells
- Never cleared → intracellular latency
  - Person infected, and potentially infectious for ever
Worldwide CMV seroprevalence rates among women of reproductive age and birth prevalence of congenital CMV infection.

2005-2014 CMV prevalence in first-time CBS donors = 42%
- Increases with age:
  → 30% 17-19 yo
  → 67% 70+ yo

O’Brien SF, et al. CSTM 2015 (poster)
Transmission

- Direct contact with infected mucosal epithelial cells during viral shedding (symptomatic or asymptomatic)
  - Urine, saliva, breast milk, cervical secretions, semen
- Blood transfusion, bone marrow transplant, solid organ transplant
- Transplacental, intrapartum
- Not via aerosols

Public Health Agency of Canada
Images: http://thumbs.dreamstime.com/z/infections-de-transmission-des-maladies-de-diffusion-de-virus-cliparts-44466539.jpg
Clinical Significance

- **Primary infection** – first-time immune response
  - Active viral shedding in secretions
  - Healthy individuals – spontaneous resolution
  - Window period 6-8 weeks before seroconversion to CMV IgG positive

- **Viral latency** – low level or absence of detection
  - Genome maintained as episomes in CD14+ blood monocytic cells and CD34+ and CD33+ marrow cells (progenitor cells, monocytes)
  - Never ‘cleared’, ongoing potential infectivity
  - Return to active replication with monocyte → macrophage differentiation

[References]
Definitions

- **CMV Infection (active)**
  - Isolation of CMV virus or viral proteins in any body fluid
  - Predominantly asymptomatic to mono-like illness

- **CMV Disease**
  - Significant morbidity and mortality
  - End-organ damage as a result of CMV infection
    - Pneumonia, gastroenteritis, hepatitis, retinitis, nephritis, cystitis, myocarditis
    - Requires a tissue specimen with CMV infiltration
      - PCR positivity insufficient (except for CNS)
  - Congenital CMV
    - Petechiae, HSM; microcephaly, cognitive delay, hearing loss

- **Primary or recurrent viral presence**
Describe CMV reduction strategies and donor testing at Canadian Blood Services
CMV DNA prevalence in donor plasma samples

- CMV seroconversion rates among healthy blood donors is 0.2-1.2%

- Estimated rate of units from newly seropositive healthy donors containing CMV DNA is 0.2-0.3%

- Window period of possible DNA presence before seroconversion
  - 6-8 weeks after primary infection
  - Free CMV DNA cannot be removed by LR, detected up to 1y after primary infection → highest concentration during this year
  - Free DNA seropositivity ≠ infectivity

Transfusion. 2003;43:314-21
Transfusion. 2007;47:1972-83
CBS Component Preparation

Buffy Coat Production Method (BCPM)

Whole Blood Filtration Method (WB)

Image from Dr. R. Skeate, CBS
## CBS: Pre-storage LR in 2015

<table>
<thead>
<tr>
<th>Variable</th>
<th>B1 RBC</th>
<th>B2 RBC</th>
<th>Buffy coat platelet</th>
<th>Apheresis platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td># tested units</td>
<td>5045</td>
<td>3401</td>
<td>1210</td>
<td>1116</td>
</tr>
<tr>
<td>WBC median</td>
<td>$0.063 \times 10^6$</td>
<td>$0.080 \times 10^6$</td>
<td>$0 \times 10^6$</td>
<td>$0 \times 10^6$</td>
</tr>
<tr>
<td>Pass rate following LR</td>
<td>99.88%</td>
<td>99.76%</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Residual WBC threshold in final LR product:
- FDA recommendation (CBS QA threshold) = $<5 \times 10^6$
- Other countries = $<1 \times 10^6$

Slide courtesy Dr. J. Callum
Donor Testing – PK7300

- Serologic testing of donor plasma sample
  - Passive Particle Agglutination assay
    - Qualitative, polyspecific total Ab detection (IgM + IgG)
    - Donor screening, results considered final

According to Beckman Coulter (EDTA Plasma)
- Sensitivity 100%
- Specificity 99.6%

Beckman Coulter PK CMV-PA System manual, Nov 2009
CBS Product Management

- CMV negative cellular components are labelled “CMV –” based on serologic result
  - No CMV label if positive or untested/unknown
  - Plasma units not identified with CMV status

- Quality Control – National Testing Lab performs residual post-LR WBC counts in cellular products monthly
  - Sampling of 1% of LR cellular products w/in 24h of processing
  - Must contain <5 x 10^6 residual WBC (flow cytometry)
Healthcare System Costs

- **Logistical challenges**
  - Delays when anti-CMV negative units not available or need to be specially ordered from CBS sites
  - Limited supply for patients with less common blood groups or in need of special products

- **Financial implications**
  - Increased outdating of standard LR-only platelet products related to CMV selection preferences
  - Annual reagent cost for Canada for testing of donor units for CMV ~ CAN $700,000.
    - Not including labour cost of testing or transportation cost for delivery

Slide courtesy Dr. M Zeller, source SickKids Memo 2012
What about CMV NAT Testing?

- Not presently performed by CBS (or elsewhere!)
  - No Health Canada approved CMV NAT

  - Largest comparative, prospective analysis of 2 validated modalities each of serologic and NAT for CMV
    - 416 seropositive, 514 seronegative by both assays; 70 discrepant
    - 2/1000 samples positive by serology and both PCR methods

- In conclusion → No firm evidence that NAT testing detects additional CMV positive donors
• Summarize the literature demonstrating that CMV-U (CMV safe) and CMV seronegative components are equivalent in safety in BMT patients

• Review the risk of transfusion-transmitted CMV in the era of pre-storage leukoreduction
Blood Product Leukoreduction (LR)

- Pre-storage LR standard practice in UK and Canada since 1999 – platelets and RBC
  - Clear reduction in CMV transmission risk
  - WBC/unit RBC:
    - no LR = $10^8$
    - pre-storage LR (present practice) = $<10^5$

- CMV-U [untested/unknown] or “CMV safe” = LR only
- CMV negative = LR + serology negative
- LR does reduce but cannot completely eliminate CMV risk due to:
  - Residual leukocytes and DNA, virions (window period)
- Viral load required to transmit infection by transfusion is unknown

SaBTO 2012 CMV Position Statement
Blood. 2004; 103:1137-39
Canadian Consensus Conference

- Prevention of Post-transfusion CMV in the Era of Universal Leukoreduction (Toronto, 2000)

Present Recommendations for provision of CMV negative (seronegative) blood in Canada (LR blood):

<table>
<thead>
<tr>
<th>Recommended:</th>
<th>Possibly recommended:</th>
<th>Not recommended:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Intrauterine transfusions</td>
<td>- CMV sero –ve solid organ transplant recipients</td>
<td>- Auto SCT recipients</td>
</tr>
<tr>
<td>- CMV sero –ve pregnant women</td>
<td>- CMV sero –ve pre-allo SCT</td>
<td>- Neonates</td>
</tr>
<tr>
<td>- CMV sero –ve allo-SCT recipients</td>
<td>- CMV sero –ve pts w/HIV</td>
<td></td>
</tr>
</tbody>
</table>
CMV Seronegative HSCT Recipients

• 4 recent studies in adults to suggest LR only is sufficient to prevent TT-CMV

   • Single center institution in Germany
   • Prospective, observational study over 10y (99-09)
   • n=143; all allo-SCT pts received pre-storage LR CMV-U RBC
   • 23 CMV neg/neg – monitored clinically, CMV NAT weekly
   • 17/23 tested positive for CMV IgG (low levels),
     • NO IgM, NO DNA by PCR detected
     • Suspected passive IgG from products only
   • Rate of confirmed TT-CMV = 0%
CMV Seronegative HSCT Recipients

[Letter]

- Single center – University of Michigan
- Retrospective analysis, prospectively collected data
- CMV neg/neg allo-BMT patients; n=100
- Transfused 3690 LR only, CMV-U components
- Rec’d RBC leukoreduced *after* storage
- Weekly CMV NAT monitoring
- **Rate of confirmed TT-CMV = 0%**
  - Negative CMV NAT, IgM
  - 2 patents CMV IgG + only – presumed passive acquisition
CMV Seronegative HSCT Recipients


- Canadian study – Ottawa, Oct 99-June 12
- Retrospective uncontrolled “before – after” study; prospective data collection
- CMV neg/neg allo-HSCT patient groups
  - Oct 99-Dec 06 → CMV LR + seronegative blood
  - Jan 07-June 12 → CMV LR blood
- Primary outcome – CMV viremia
  - PCR positive
- Weekly CMV PCR monitoring
CMV Seronegative HSCT Recipients

- n=89 LR + CMV neg
- n=77 LR only
- CMV viremia, PCR positive n=4:
  - 1 LR only (1.3%)
  - 3 LR + CMV neg (3.4%)
    - p=0.6244
  - 2/3 LR + CMV neg pts developed CMV disease

- No difference in secondary outcomes

No difference in CMV viremia or disease in patients receiving LR + CMV neg versus leukoreduced-only blood components
CMV Seronegative HSCT Recipients

   - Two UK sites – Oxford, Birmingham
   - Retrospective analysis of TT-CMV
   - CMV neg/neg allo-BMT patients; n=76
   - Followed weekly for CMV DNA
   - Transfused with 1442 LR-only components with 1862 donor exposures
   - Rate of confirmed TT-CMV = 0%
Systematic Review of Clinical Studies

- Studies with comparison groups of blood component CMV reduction strategies for at-risk patient populations → BMT and VLBWI
  - No relevant studies found for SOT, pregnant women, 1° immune deficiency
- 10 studies included in the meta-analysis
  - 6 observational with 901 pts; 4 RCTs with 680 pts
- 7 chemo/allo-BMT; 3 infant studies
  - 2 allo-BMT studies “modern” with pre-storage leukoreduction (595 pts; published 2003, 2013)
  - 2 allo-BMT looked at LR vs LR + CMV seronegative
  - No attempt to link donor to recipient
- Infant studies problematic as infants fed CMV-infected breast milk included

Mainou et al. Transfusion 2016; epub ahead of publication
• No significant difference in clinical or laboratory CMV infection
• Cannot favor one CMV reduction strategy over another
  • LR only vs LR + CMV seronegative

Mainou et al. Transfusion 2016; epub ahead of publication
Incredibly low probabilities of TT-CMV in the pre-storage LR era

<table>
<thead>
<tr>
<th>Product</th>
<th>Probability</th>
<th>95% confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>1 in 7,790,000</td>
<td>1 in 771,307,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 in 993,000</td>
</tr>
<tr>
<td>Platelets</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 in 1,074,000</td>
</tr>
<tr>
<td>Combined</td>
<td>1 in 13,575,000</td>
<td>1 in 1,344,167,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 in 1,730,000</td>
</tr>
</tbody>
</table>

Calculations based on risk of LR filter failure \( p(f) \) and donor concurrently being CMV infectious \( p(viraemia) \)

\[
RBC \text{ unit } [p(\text{Inf})] = p(f) \times p(viraemia) \\
= 0.001083 \times 0.0011850538 \\
= 1.2837 \times 10^{-7} \text{ (95\% CI: } 1.297 \times 10^{-9} - 1.007 \times 10^{-6}) \text{ or,} \\
1 \text{ in } 7789519 \text{ (95\% CI: } 1 \text{ in } 771306874 - 1 \text{ in } 992979) .
\]

Seed et al, *Vox Sang* 2015; 109: 11-17
Criticism of infectivity estimates

• Exclusion of infections from cell free DNA…but the authors argued:
  ◦ Theoretical only
  ◦ No reported cases of CMV transmission by plasma transfusion
  ◦ ‘Dose’ of viral DNA to cause infection unknown
  ◦ Cell free DNA is highly fragmented
  ◦ Studies in mice fail to demonstrate any infectivity

Seed et al, *Vox Sang* 2015; 109: 11-17
Transfusion Related CMV Risk

Warm whole blood 1 in 2

Refrigerated whole blood 1 in 100

Seronegative non-LR 1 in 66,000

Pre-storage LR only 1 in 13,575,000

Seed et al. Vox Sang 2015; 109: 11-17
Allain et al. Biologicals 2009; 37: 71-77
Logistical Issue in BMT Recipients

• Passive CMV IgG transmission does occur from transfusion

• For the most reliable recipient CMV status, CMV Ab testing MUST be drawn before the first platelet transfusion for potential HSCT and SOT patients

• Observational study of 31 HSCT patients
  ◦ 35.5% did not have their CMV Ab checked before the first transfusion
  ◦ 93.5% had multiple CMV Ab tests pre-transplant
  ◦ 27.6% had “flipping” results suggesting passive Ab detection
  ◦ 1 CMV-neg donor had a CMV-pos donor selected in error

Clinical monitoring in HSCT patients

- Important because of pre-transplant CMV status and CMV safe transfusion
- Aggressive CMV monitoring and pre-emptive treatment has resulted in a significant reduction of CMV disease in allo-HSCT patients
  - 20-30% \rightarrow <5%
  - Standard practice since 1990s

*Blood, 2013, 122: 3359-54*
CMV seronegative red cells and platelets may be replaced with leukodepleted blood components for adults and children post HSCT for all patient groups.

Patients requiring transfusions who may require a transplant in the future may also safely be transfused with leukodepleted products.

CMV PCR monitoring should be considered for all patients to allow early detection of any possible CMV infection.

• CMV (cytomegalovirus) Disease Prevention
  ◦ Monitoring and pre-emptive therapy by quantitative plasma PCR all allogeneic BMT patients, regardless of serostatus
  ◦ q weekly x 100 days, then q monthly x 1yr
  ◦ If >25,000 IU/mL, treat pre-emptively with ganciclovir or valganciclovir twice daily.

• Blood products
  ◦ For all recipients, use CMV safe blood products (leukocyte depleted, irradiated)
Canadian National Advisory Committee on Blood and Blood Products (20.04.14)

Statement regarding appropriateness of use of CMV sero negative vs CMV safe product

- The NAC recommends that CMV safe and CMV IgG seronegative products be considered equivalent for the majority of patient populations including:
  - Adult and pediatric hematopoietic stem cell recipients,
  - CMV seronegative patients who may require future transplant, and
  - Immunodeficient patients
Cellular blood components should be selected from CMV negative donors or processed (using a method such as leukoreduction) to reduce the risk of CMV transmission in the following situations:

a) all intrauterine transfusions; and

b) infant transfusions when the recipient weighs less than 1200 g at birth and the infant or the mother is CMV antibody-negative (or unknown).
### Current Canadian Local Practices

<table>
<thead>
<tr>
<th>Adult Allo BMT Center</th>
<th>CMV status of transfusions to BMT recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancouver General Hospital (BC)</td>
<td>CMV Neg to D-/R-</td>
</tr>
<tr>
<td>Foothills Medical Center (AB)</td>
<td>CMV-U</td>
</tr>
<tr>
<td>Royal University Hospital (SK)</td>
<td>CMV Neg to D-/R-</td>
</tr>
<tr>
<td>Princess Margaret Hospital (ON)</td>
<td>CMV-U</td>
</tr>
<tr>
<td>The Ottawa Hospital (ON)</td>
<td>CMV-U</td>
</tr>
<tr>
<td>London Health Sciences Center (ON)</td>
<td>CMV-U</td>
</tr>
<tr>
<td>McMaster University Medical Center (ON)</td>
<td>CMV-U</td>
</tr>
<tr>
<td>McGill University Health Center (QC)</td>
<td>CMV-U</td>
</tr>
<tr>
<td>QEII Health Sciences Center (NS)</td>
<td>CMV Neg to D-/R-</td>
</tr>
</tbody>
</table>

*CMV-U = CMV untested (pre-storage leukodepleted only)*
Conclusion

- Current evidence demonstrates that CMV seronegative components are equivalent in safety to CMV-U (CMV safe) components in terms of transfusion related CMV infection risk.

- **Proposal:**
  
  *Transfusion medicine shall provide CMV-U (CMV safe) components only for all adult BMT patients, regardless of recipient pre-transplant CMV serostatus.*
Discussion and Questions