Cytomegalovirus (CMV) Viral Load in Bronchoalveolar Lavage Fluid (BALF) and Plasma to Diagnose Lung Transplant Associated CMV Pneumonia

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Background

- Cytomegalovirus (CMV) infection is a common complication to lung transplantation, and can progress to potentially life threatening CMV pneumonia\(^1\)

- Routine care for screening and monitoring of CMV infection following lung transplantation is by CMV PCR in plasma\(^1\)

- CMV PCR on bronchoalveolar lavage fluid (BALF) retrieved through bronchoscopy has evolved as a supplement to routine screening in plasma

- The relationship between CMV PCR viral load in BALF and plasma is uncertain and highly variable\(^2,3\), and the optimal diagnostic tool for CMV pneumonia remains controversial\(^4\)

2. Razonable RR et al, CID, 2013
Aim of the study

- Among lung transplant recipients with positive CMV PCR in BALF
  - Investigate the association between BALF CMV viral load and presence of CMV pneumonia
  - Study whether the association was influenced by co-existing pulmonary infections or rejection
  - Establish the optimal BALF CMV viral load cut-off associated with CMV pneumonia
  - Determine the correlation between CMV PCR in plasma and in BALF, and the use of plasma CMV to diagnose CMV pneumonia
Patients

- Recipients of lung transplantation transplanted between January 2010 to March 2015 at Rigshospitalet, Denmark
  - The lung tx program did routine bronchoscopy at regular intervals during 1st year post-transplantation; BALF examined by CMV PCR
- In these recipients, we focused on those who:
  - had a known CMV IgG serostatus of the donor (D) and recipient (R) at transplantation
    - Possible combinations: D+/R+, D+/R-, D-/R+
  - had ≥ 1 positive CMV PCR detected in BALF within the first year of transplantation
    - Lower limit of detection: 300 copies/mL (=270 IU/mL)
Study design

• Each CMV PCR positive BALF episode was treated as a separate episode

• Two physicians assessed the episodes for CMV pneumonia from medical records based on the consensus definition of CMV pneumonia used at our hospital
<table>
<thead>
<tr>
<th>Certainty of diagnosis</th>
<th>Criteria for diagnosis of CMV pneumonia in lung transplant recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proven</td>
<td>Relevant symptoms/signs of pneumonia + positive CMV histology in lung biopsy</td>
</tr>
<tr>
<td>Probable</td>
<td>Relevant symptoms/signs of pneumonia + positive CMV PCR in BALF + infiltrative changes on CT-scan (if available)</td>
</tr>
</tbody>
</table>

*: sub grouped according to presence of possible additional/competing cause(s)
Statistical analyses

• The optimal cut-offs for diagnosis of CMV pneumonia for CMV PCR in plasma and BALF were determined using receiver operating characteristics (ROC)

• CMV PCR viral load in plasma and BALF were correlated

• Results were unaffected by sensitivity analyses after excluding cases of the same recipient taken within four weeks
Patient population according to CMV PCR in BALF

• A total of 141 recipients were transplanted during the study period
  – a total of 981 bronchoscopies were performed in this population in 1st year post-transplant
• 66 (47%) recipients had ≥ 1 CMV PCR positive BALF episode
  – Age, gender and distribution of CMV IgG sero-constellations were evenly distributed
• Among these 66 recipients, a total of 145 CMV PCR positive BALF were detected in the first year after transplantation
  – For each of these 145 episodes, symptoms and signs fulfilling diagnosis of pneumonia were ascertained
Prevalence of CMV pneumonia and presence of pulmonary co-pathogens in episodes with and without pneumonia

Red colour indicates co-infection*

Blue colour indicates no co-infection

* Information on positive microbiology +/- 7 days within BALF was collected from medical records, and type of co-pathogen/s documented (fungal/bacterial/viral)
CMV pneumonia episodes by degree of certainty of diagnosis and presence of pulmonary co-pathogens

% of total CMV PCR positive BALF

Red colour indicates co-infection

Blue colour indicates no co-infection

Proven CMV Pneumonia (n=9)
Probable CMV Pneumonia (n=25)

BALF CMV PCR episodes fulfilling CMV pneumonia definition (n=34)
Comparison of BALF CMV viral load in episodes with and without CMV pneumonia

Median (IQR) CMV viral load in BALF (log$_{10}$ copies/mL)

No pneumonia, n= 111

Pneumonia, n=34

p <0.0001

LLOD
Comparison of BALF CMV viral load in episodes with and without CMV pneumonia, stratified for competing causes.

Comparison of BALF CMV viral load in episodes with and without CMV pneumonia, stratified for competing causes.

**Circles:** BALF CMV PCR episodes without CMV Pneumonia

**Diamonds:** BALF CMV PCR episodes with CMV Pneumonia

**Median (IQR) CMV viral load in BALF (log\textsubscript{10} copies/mL)**

<table>
<thead>
<tr>
<th>No of episodes</th>
<th>N=29</th>
<th>N=12</th>
<th>N=45</th>
<th>N=12</th>
<th>N=49</th>
<th>N=20</th>
<th>N=62</th>
<th>N=14</th>
<th>N=27</th>
<th>N=6</th>
<th>N=111</th>
<th>N=34</th>
</tr>
</thead>
</table>

**p <0.0001**
Correlation of CMV viral load measured in BALF and in plasma

Correlation 55%,
p < 0.0001

- Dashed green lines indicate lower limit of detection (LLOD) of CMV PCR kit at 300 copies/mL (=270 IU/mL)
Correlation of CMV viral load measured in BALF and in plasma with CMV PCR

- Dashed green lines indicate lower limit of detection (LLOD) of CMV PCR kit at 300 copies/mL (=270 IU/mL)

Correlation 55%,
p < 0.0001
Correlation of CMV viral load measured in BALF and in plasma with CMV PCR

Correlation 55%, $p < 0.0001$

In 37% of episodes with CMV pneumonia, the corresponding plasma CMV PCR test was negative.

- Dashed green lines indicate lower limit of detection (LLOD) of CMV PCR kit at 300 copies/mL (=270 IU/mL)
Diagnostic accuracy of CMV PCR in plasma to diagnose CMV pneumonia

<table>
<thead>
<tr>
<th>Cut off for virus load in plasma with CMV PCR (copies/mL)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>305</td>
<td>63</td>
<td>76</td>
</tr>
<tr>
<td>1,025</td>
<td>40</td>
<td>98</td>
</tr>
<tr>
<td>9,750</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

Area Under the Curve: 76%
Diagnostic accuracy of CMV PCR in BALF to diagnose CMV pneumonia

Cut off for virus load in BALF with CMV PCR (copies/mL) | Sensitivity (%) | Specificity (%)
--- | --- | ---
1,450 | 100 | 50
5,050 | 91 | 77
6,550 | 79 | 80
17,500 | 65 | 89

Area Under the Curve: 90%
Conclusions

• CMV viral load in BALF was higher for episodes representing CMV pneumonia regardless of presence of competing causes

  – A large proportion of the episodes with positive CMV PCR in BALF have concurrent co-infection/s

  – In case of suspected CMV pneumonia, BAL with CMV PCR and investigation of co-pathogens with convention methods are advisable
• CMV PCR in plasma was negative in 37% of the CMV pneumonia episodes, and had a poor ability to diagnose CMV pneumonia
  – Thus, a negative plasma CMV PCR cannot alone rule out CMV pneumonia

• CMV PCR in BALF showed a high diagnostic accuracy for diagnosis of CMV pneumonia

• Our results provide rationale for expanding the use of CMV PCR in BALF for earlier diagnosis of CMV pneumonia in lung recipients
Acknowledgements

• Thank you to all co-authors involved in the project:
  – H.H. Schultz, J.U. Jensen, C. Andersen, M. Perch, J.D. Lundgren, M. Iversen
Back-up slides
CMV PCR in BALF

• Patients were routinely screened with bronchoscopy,* collecting biopsies and BALF in the first year following transplantation
  • investigated with CMV immunohistochemistry and CMV PCR respectively

• Furthermore, all BALF from bronchoscopies performed by indication were investigated with CMV PCR

*Surveillance bronchoscopy with trans bronchial biopsies (TBB) and BAL performed at week 2, 4, 6, 12, 26 and 52 post transplant
Clinical features of 145 CMV PCR positive BALF in 67 lung recipients according to presentation of CMV pneumonia

<table>
<thead>
<tr>
<th>Characteristics at BALF</th>
<th>NO</th>
<th>CMV PNEUMONIA?</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Overall, n=111</td>
<td>Rejection, n=29</td>
</tr>
<tr>
<td>% of BALF with biopsy*</td>
<td>67%</td>
<td>74/111</td>
</tr>
<tr>
<td>% with CMV detected in biopsy</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>% with acute rejection in biopsy</td>
<td>39%</td>
<td>29/74</td>
</tr>
<tr>
<td>% with interstitial changes in biopsy**</td>
<td>44.5%</td>
<td>33/74</td>
</tr>
<tr>
<td>% of BALF where CMV is the only detected pathogen**</td>
<td>56%</td>
<td>62/111</td>
</tr>
<tr>
<td>Co-infections***</td>
<td>34%</td>
<td>38/111</td>
</tr>
<tr>
<td>% with fungal co-infection</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>% with bacterial co-infection</td>
<td>21%</td>
<td>23/111</td>
</tr>
<tr>
<td>% with viral co infection</td>
<td>9%</td>
<td>10/111</td>
</tr>
<tr>
<td>Median time from tx to BALF (IQR)</td>
<td>169 (89-216)</td>
<td>175 (90-214)</td>
</tr>
<tr>
<td>Median VL in BALF CMV PCR (IQR)</td>
<td>1,400 (500-4,700)</td>
<td>830 (500-2,500)</td>
</tr>
<tr>
<td>Proportion of BALF with CMV PCR in plasma****</td>
<td>86%</td>
<td>96/111</td>
</tr>
<tr>
<td>% with positive CMV PCR</td>
<td>24%</td>
<td>23/96</td>
</tr>
<tr>
<td>Median VL in positive plasma CMV PCR (IQR)</td>
<td>400 (300-730)</td>
<td>1,000 (400-1,800)</td>
</tr>
</tbody>
</table>

*Grading: BOOP, DAD. **Detected within +/- 7 days from the BAL fluid. ***Detected within +/- 7 days from the BAL fluid. Note that some patients have > 1 co-infections at the same time. ****Measured in plasma with CMV PCR +/- 7 days from the BAL fluid.

N.A. = Not applicable or not available.