

# **CMV PNEUMONIA IN IMMUNOCOMPROMISED PATIENTS IN ICU- DIAGNOSIS**

Dr Jayabharathi Palanivel

# IMMUNOCOMPROMISED PATIENTS -SUBGROUPS

- 1) Solid organ transplant recipients
- 2) Hematopoietic cell transplant recipients
- 3) Human immunodeficiency virus
- 4) Patient with Immunomodulating drugs/ chemotherapy

### QUESTION 1

How to diagnose ?

CMV infection vs  
disease ?

### QUESTION 2

When to treat ?

### QUESTION 3

Whom to treat?

# Challenges in diagnosis

- Nonspecific imaging: GGO, interstitial infiltrates overlap with other infections/ILD.
- Infection vs disease dilemma: CMV detection  $\neq$  active pneumonitis; co-infections/confounders common.
- Diagnostic gaps : BAL CMV PCR and Serum CMV PCR lacks standardized cutoffs, assays vary across centres
- Lung biopsy – gold standard but feasibility is less in mechanically ventilated patients
- Limited access to BAL PCR quantitative PCR / IHC in resource limited settings
- No single definitive test: Diagnosis relies on a syndromic approach (clinical + labs + imaging + exclusion).

# Therapeutic gaps

- Novel antivirals (letermovir, maribavir) are not universally available or affordable
- Drug resistance testing (UL97/UL54 genotyping) is not routine in most centres
- Guideline implementation issues: Indian ICUs adapt western guidelines but face local cost/availability constraints
- Lack of standardized ICU-specific protocols → variable practice

# DIAGNOSTIC CRITERIA- DEFINITIONS

# Definitions of Cytomegalovirus Infection and Disease in Transplant Recipients

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- **CMV Infection:** Detection of CMV replication regardless of symptoms , by virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen
- **CMV Disease:** CMV infection with attributable symptoms or signs of illness.
- **CMV Pneumonia:** CMV pneumonia is defined by the presence of signs and/or symptoms of pulmonary disease combined with the detection of CMV in bronchoalveolar lavage fluid or lung tissue samples. Detection of CMV should be performed by virus isolation, histopathologic testing, immunohistochemical analysis, or in situ hybridization

## Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials

Per Ljungman,<sup>1,2</sup> Michael Boeckh,<sup>3,4</sup> Hans H. Hirsch,<sup>5</sup> Filip Josephson,<sup>1</sup> Jens Lundgren,<sup>7</sup> Garrett Nichols,<sup>8</sup> Andreas Piskis,<sup>9</sup> Raymond R. Razonable,<sup>10</sup> Veronica Miller,<sup>11</sup> and Paul D. Griffiths<sup>12</sup>; for the Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum\*

<sup>1</sup>Departments of Allogeneic Stem Cell Transplantation and Hematology, Karolinska University Hospital, Solna; <sup>2</sup>Division of Hematology, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm; and <sup>3</sup>Swedish Medical Products Agency, Uppsala, Sweden; <sup>4</sup>Vaccine and Infectious Disease and Clinical Research Division, Fred Hutchinson Cancer Research Center; and <sup>5</sup>Department of Medicine, University of Washington, Seattle; <sup>6</sup>Department of Biomedicine, University of Basel, Switzerland; <sup>7</sup>Centre for Health and Infectious Disease Research (CHIR), Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Denmark; <sup>8</sup>Chimerix, Inc, Durham, North Carolina; <sup>9</sup>Division of Antiviral Products, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland; <sup>10</sup>Division of Infectious Diseases, Department of Medicine, William J. von Liebig Center for Transplantation and Clinical Regeneration, Mayo Clinic, Rochester, Minnesota; <sup>11</sup>Forum for Collaborative HIV Research, University of California, Berkeley; and <sup>12</sup>Institute for Immunity and Transplantation, University College London Medical School, United Kingdom

### Cytomegalovirus Disease Categories and Required Quality of Evidence

| Disease                    | Proven | Probable | Possible |
|----------------------------|--------|----------|----------|
| Pneumonia                  | Yes    | Yes      | Yes      |
| Gastrointestinal disease   | Yes    | Yes      | Yes      |
| Hepatitis                  | Yes    | No       | No       |
| Retinitis                  | Yes    | No       | No       |
| Encephalitis/ventriculitis | Yes    | Yes      | No       |
| Nephritis                  | Yes    | No       | No       |
| Cystitis                   | Yes    | No       | No       |
| Myocarditis                | Yes    | No       | No       |
| Pancreatitis               | Yes    | No       | No       |
| Other end-organ diseases   | Yes    | No       | No       |
| Syndrome                   | No     | Yes      | No       |

All 3 categories require appropriate clinical symptoms and/or signs.

- The main changes were to introduce a “probable disease” category and to incorporate nucleic acid testing (NAT) in some end-organ disease categories.
- Proven CMV disease requires definitive tissue evidence for all categories
- while probable disease classification is limited to pneumonitis and gastrointestinal disease.
- Possible disease applies only to pneumonitis

# CMV PNEUMONITIS

## Proven

Clinical symptoms / signs of pneumonia (e.g., hypoxia, imaging findings), and CMV documented in lung tissue by virus isolation and histopathology or IHC

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## Probable

Clinical symptoms/signs + CMV in BAL fluid by viral isolation or CMV DNA quantitation - the threshold for CMV DNA load in BAL fluid is not established.

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## Possible

clinical + radiologic features, CMV DNA detection in BAL is borderline / lower, where there are co-pathogens present ,making attribution uncertain.

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## Consensus Definitions of Cytomegalovirus (CMV) Infection and Disease in Transplant Patients Including Resistant and Refractory CMV for Use in Clinical Trials: 2024 Update From the Transplant Associated Virus Infections Forum

Per Ljunggren,<sup>1,2,3</sup> Roy F. Chensyal,<sup>4</sup> Farouk Khawaja,<sup>5</sup> Sophie Alain,<sup>6</sup> Robin Avery,<sup>7</sup> Cyrus Badshah,<sup>8</sup> Michael Bosch,<sup>1,9</sup> Martha Fournier,<sup>4</sup> Aimee Hodowanec,<sup>10</sup> Takashi Kosztka,<sup>11</sup> Ajit P. Limaye,<sup>12</sup> Dial Mousal,<sup>13</sup> Yoichiro Natori,<sup>14</sup> David Navarro,<sup>15,16</sup> Andreas Pfkir,<sup>17</sup> Raymond R. Razonable,<sup>18,19</sup> Gabriel Weizman,<sup>18,20</sup> Veronica Miller,<sup>21</sup> Paul D. Griffiths,<sup>22</sup> and Cassile N. Kotton<sup>23</sup>; for the CMV Definitions Working Group of the Transplant Associated Virus Infections Forum

- Definition not changed- Proven , Probable and Possible
- Introduced Refractory and resistant CMV infection
- CMV syndrome applicable only to SOT recipients.

- Possible CMV Pneumonia- controversial

The detection of a concomitant primary airway pathogen together with a borderline level of CMV DNA in BAL fluid decreases the likelihood of the pneumonia being due to CMV, while a high CMV viral load in BAL fluid together with another opportunistic pathogen increases the likelihood of CMV pneumonia.

### Definition of Probable Cytomegalovirus (CMV) Syndrome in Solid Organ Transplant Patients Based on Clinical and Laboratory Criteria (at Least 2 Criteria are Required) and Detection of CMV DNA or Antigen in Whole Blood or Plasma Within 1 Week of Symptoms

- a. Fever  $\geq 38^{\circ}\text{C}$  for at least 2 days of which at least 1 measurement is documented in a healthcare setting and without another identified cause of the fever
- b. New or increased malaise CTCAE toxicity grade 2, including muscle aches or general achiness, headache, or new or increased fatigue (CTCAE toxicity grade 3)
- c. A WBC count of  $< 3500/\mu\text{L}$  if the WBC count prior to the development of clinical symptoms was  $\geq 4000/\mu\text{L}$  or a WBC decrease of  $> 20\%$  if the WBC count prior to the development of clinical symptoms was  $< 4000/\mu\text{L}$ ; the corresponding neutrophil counts are  $< 1500/\mu\text{L}$  or a decrease of more than 20% if the neutrophil count before the onset of symptoms was below  $1500/\mu\text{L}$
- d.  $\geq 5\%$  atypical lymphocytes
- e. A platelet count of  $< 100\,000/\mu\text{L}$  if the platelet count prior to the development of clinical symptoms was  $\geq 115\,000/\text{mL}$  or a decrease of  $> 20\%$  if the platelet count prior to the development of clinical symptoms was  $< 115\,000/\mu\text{L}$
- f. Elevation of hepatic transaminases (alanine aminotransferase or aspartate aminotransferase) to  $> 2 \times$  upper limit of normal or  $> 2 \times$  baseline value (if abnormal at baseline); baseline defined as last value before cytomegalovirus viremia was documented (applicable to non-liver transplant recipients)

### Refractory CMV infection

CMV viremia that either (a) increases by  $>1 \log_{10}$  after  $\geq 2$  weeks of appropriate therapy, or (b) persists ( $\leq 1 \log_{10}$  increase or decrease) after the same period

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### Refractory CMV End-Organ Disease

Worsening signs and symptoms or progression to end-organ disease if initial disease not present, OR lack of improvement in signs and symptoms after  $\geq 2$  weeks therapy.

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### Resistant CMV Infection

Refractory + viral genetic alteration that decrease susceptibility to 1 or more antiviral drugs

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# DIAGNOSTIC TEST

- PCR - serum and BAL
- Antigenemia
- Culture
- Imaging

# CMV PCR

Serum

# Interpreting Quantitative Cytomegalovirus DNA Testing: Understanding the Laboratory Perspective

Colleen S. Kraft,<sup>1,2</sup> Wendy S. Armstrong,<sup>2</sup> and Angela M. Caliendo<sup>1,2</sup>

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- There are currently no FDA–approved laboratory tests for the quantifying CMV DNA.
- CMV viral load tests are considered laboratory-developed tests (LDTs) , validated by an individual labs.
- Cut off values vary among institutions due to lack of standardization and LDT performance
- International standard for CMV was developed by WHO in 2010 to allow labs to assess accuracy and calibrate LDTs
- Results are now expressed in IU/mL instead of copies/mL, improving comparability across assays.

# Analysis performance and clinical interpretation

- Test methods differ in nucleic acid extraction , primer selection and test chemistry affecting viral load values
- Quantitative CMV testing is typically performed on whole blood or plasma, each having strengths and limitations .
- Performance characteristics include the limit of detection (LOD), lower and upper LOQ.
- Linear range of CMV viral load tests is usually at least 6 log 10
- Trends in viral load over time are often more informative than single measurements.

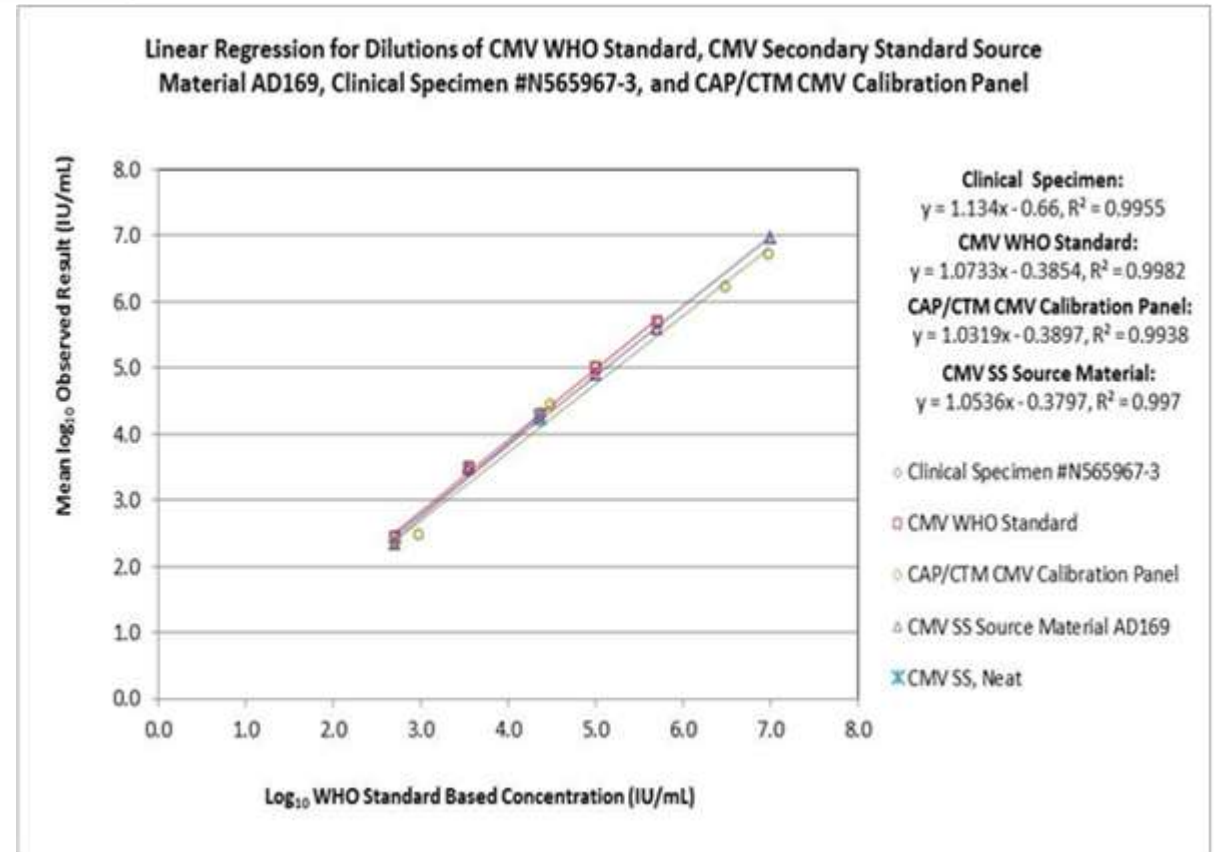
# An International Multicenter Performance Analysis of Cytomegalovirus Load Tests

Hans H. Hirsch,<sup>1</sup> Irmeli Lautenschlager,<sup>2</sup> Benjamin A. Pinsky,<sup>3</sup> Laura Cardenoso,<sup>4</sup> Shagufta Aslam,<sup>5</sup> Bryan Cobb,<sup>5</sup> Regis A. Vilchez,<sup>5,a</sup> and Alexandra Valsamakis<sup>5</sup>

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Figure showing the Test for co-linearity between 1st WHO International Standard and Standards used in calibration and standardization of the CAP/CTM CMV Test.

The linear regression shows very high agreement ( $R^2 \approx 0.99$ ) between the WHO CMV standard and multiple clinical/reference materials, confirming that assays calibrated to the WHO standard give reliably proportional viral load measurements in IU/mL across different sample types



# Comparison of Standardized Cytomegalovirus (CMV) Viral Load Thresholds in Whole Blood and Plasma of Solid Organ and Hematopoietic Stem Cell Transplant Recipients with CMV Infection and Disease

M. Veronica Divoerti,<sup>1,4</sup> Brian D. Lahr,<sup>2</sup> Jeffrey J. Germer,<sup>3</sup> Joseph D. Yao,<sup>3</sup> Michelle L. Gartner,<sup>3</sup> and Raymond R. Razonable<sup>4,5</sup>

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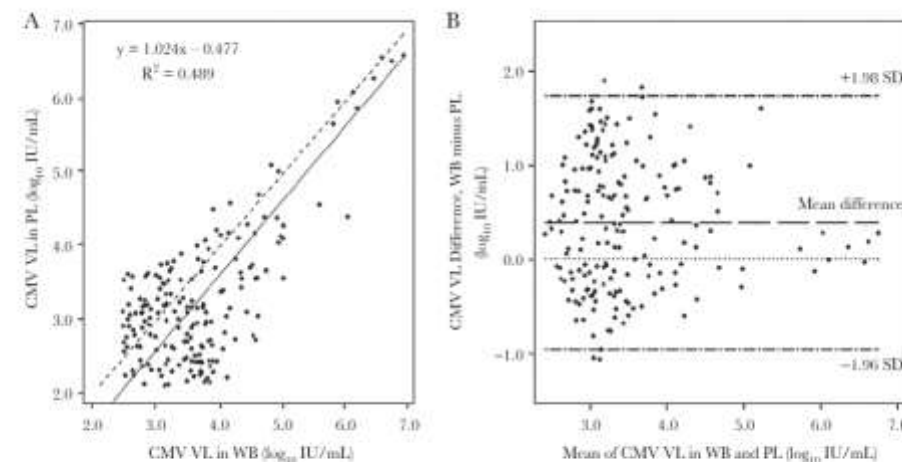
**Categorical Agreement Between CMV VL in WB and PL Among All Patient Groups**

| CMV VL in PL             | CMV VL in WB        |                          |              | Overall Agreement (%) | Kappa (95% CI)   |
|--------------------------|---------------------|--------------------------|--------------|-----------------------|------------------|
|                          | Target Not Detected | Detected, but <822 IU/mL | Quantifiable |                       |                  |
| Target not detected      | 72 (17.9%)          | 9 (2.2%)                 | 0 (0.0%)     | 279/403 (69.2%)       | 0.49 (0.43–0.56) |
| Detected, but <137 IU/mL | 44 (10.9%)          | 22 (5.5%)                | 5 (1.2%)     |                       |                  |
| Quantifiable             | 17 (4.2%)           | 49 (12.2%)               | 185 (45.9%)  |                       |                  |

The Kappa = 0.49 suggests that while the two methods are reasonably aligned, they are not interchangeable.

The study prospectively measured CMV viral load in plasma and whole blood samples from transplant recipients with CMV infection and disease.

Samples were tested using the CAP/CTM CMV assay



Correlation and agreement of CMV VL results from PL and WB as determined by Deming regression analysis (A) and Bland-Altman plot (B). Dashed line in the regression plot represents the line of unity. Abbreviations: CMV, cytomegalovirus; PL, plasma; VL, viral load; WB, whole blood.

### Comparison of Initial and Peak CMV VL in PL and WB Based on Symptomatic and Asymptomatic Disease

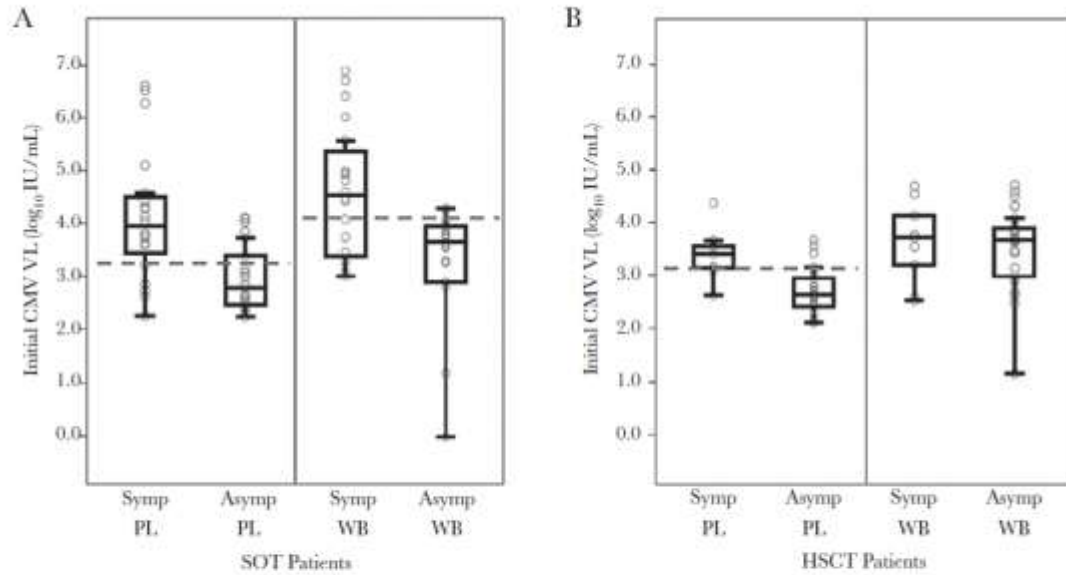
| Patient Group        | Overall Median VL (IU/mL) <sup>a</sup> | Symptomatic |                                | Asymptomatic |                                | PValue <sup>b</sup> |
|----------------------|--|-------------|--------------------------------|--------------|--------------------------------|---------------------|
|                      |  | N           | Median VL (IU/mL) <sup>a</sup> | N            | Median VL (IU/mL) <sup>a</sup> |                     |
| <b>All Patients</b>  |  |             |                                |              |                                |                     |
| Initial VL, PL       | 954 (363, 4880)                        | 29          | 4880 (1570, 20800)             | 54           | 470 (290, 1140)                | <.001               |
| Initial VL, WB       | 5480 (1740, 18200)                     | 29          | 14800 (1990, 85400)            | 54           | 5000 (1040, 8250)              | .005                |
| Peak VL, PL          | 1380 (442, 5690)                       | 31          | 5450 (1570, 20800)             | 57           | 825 (363, 2030)                | <.001               |
| Peak VL, WB          | 6240 (2020, 22400)                     | 31          | 27600 (2900, 100000)           | 57           | 5350 (1630, 11600)             | .002                |
| <b>SOT Patients</b>  |  |             |                                |              |                                |                     |
| Initial VL, PL       | 1700 (428, 12500)                      | 20          | 9100 (2830, 32200)             | 23           | 606 (297, 2530)                | <.001               |
| Initial VL, WB       | 6280 (1974, 30300)                     | 20          | 35200 (2440, 237000)           | 23           | 4520 (779, 8870)               | .004                |
| Peak VL, PL          | 2280 (434, 13400)                      | 22          | 9100 (1700, 26300)             | 24           | 863 (355, 3960)                | .003                |
| Peak VL, WB          | 7144 (2050, 36600)                     | 22          | 35200 (5080, 101000)           | 24           | 5271 (1210, 9130)              | .002                |
| <b>HSCT Patients</b> |  |             |                                |              |                                |                     |
| Initial VL, PL       | 537 (302, 1550)                        | 9           | 2790 (1530, 3870)              | 31           | 466 (281, 954)                 | <.001               |
| Initial VL, WB       | 5190 (1280, 9930)                      | 9           | 5550 (1740, 14800)             | 31           | 5130 (1040, 8250)              | .734                |
| Peak VL, PL          | 1120 (449, 3230)                       | 9           | 3220 (1570, 4880)              | 33           | 825 (414, 1900)                | .016                |
| Peak VL, WB          | 5520 (1740, 17900)                     | 9           | 6200 (1740, 37900)             | 33           | 5350 (3190, 15000)             | .490                |

Abbreviations: CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplant; PL, plasma; SOT, solid organ transplant; VL, viral load; WB, whole blood.

<sup>a</sup>Numbers in parenthesis represent the 25th and 75th percentile values (ie, interquartile range).

<sup>b</sup>Wilcoxon rank-sum test.

Among all patients, symptomatic ones have much higher initial and peak VLs in both plasma and whole blood than asymptomatic ones.



Boxplots of initial cytomegalovirus (CMV) viral load (VL) in plasma (PL) and whole blood (WB) among (A) solid organ transplant and (B) hematopoietic stem cell transplant recipients. Upper and lower bars on lines extending from each box represent maximum and minimum limits, respectively, of the result range. Top of each box indicates the third quartile; the horizontal line in the middle of each box indicates the median; the bottom of each box indicates the first quartile. Dashed lines represent the retrospectively determined VL thresholds for PL and WB that best distinguish between patients with symptomatic CMV disease and those with asymptomatic infection. Abbreviations: Asymp, asymptomatic; CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplant; PL, plasma; SOT, solid organ transplant; Symp, symptomatic; VL, viral load; WB, whole blood.

For solid organ transplant (SOT) recipients (Panel A):

Plasma (PL): 1700 IU/mL (3.23 log<sub>10</sub> IU/mL), with sensitivity of 80% and specificity of 74%  
For WB, a threshold of 12 400 IU/mL (4.09 log<sub>10</sub> IU/mL) with sensitivity of 65% and specificity of 87%

For hematopoietic stem cell transplant (HSCT) recipients (Panel B):

Plasma (PL): 1350 IU/mL, 3.13 log<sub>10</sub> IU/mL) in PL with sensitivity of 89% and specificity of 87%

Whole blood (WB): No threshold identified due to lack of significant difference between symptomatic and asymptomatic VL levels

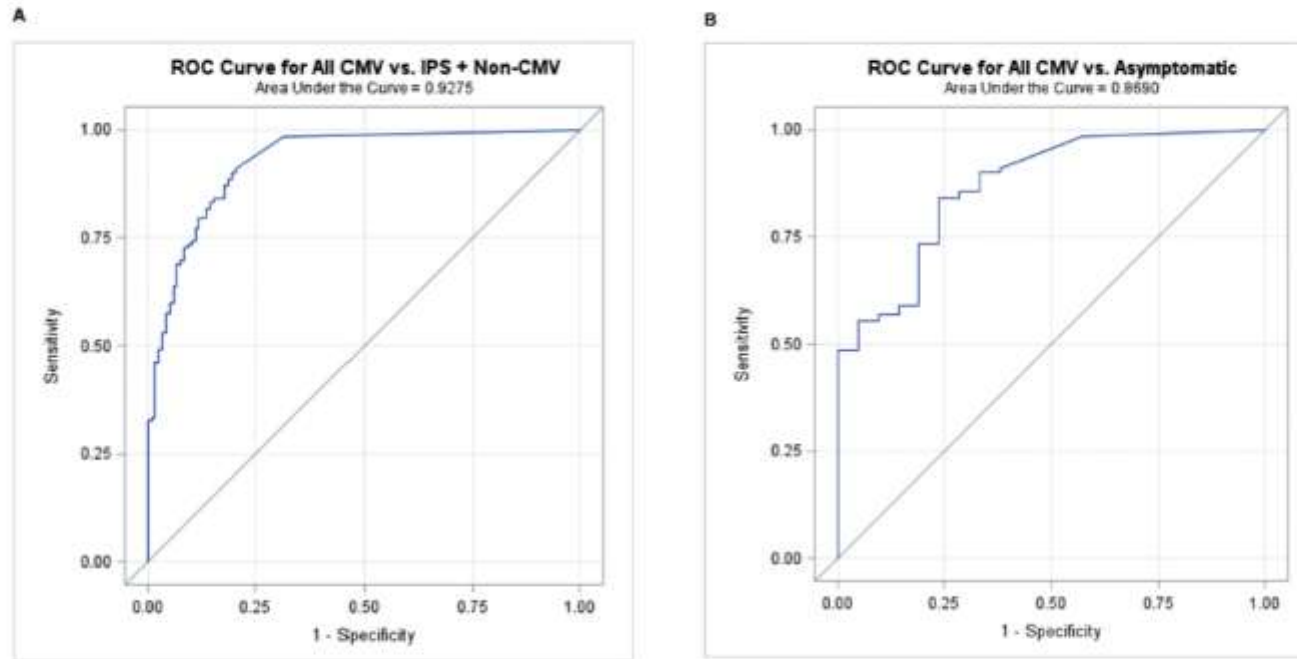
# CMV PCR

BAL

# Cytomegalovirus (CMV) DNA Quantitation in Bronchoalveolar Lavage Fluid From Hematopoietic Stem Cell Transplant Recipients With CMV Pneumonia

Michael Boeckh,<sup>1,2,3</sup> Terry Stevens-Ayers,<sup>1</sup> Giovanna Travi,<sup>1,4</sup> Meei-Li Huang,<sup>1,5</sup> Guang-Shing Cheng,<sup>2,3</sup> Hu Xie,<sup>2</sup> Wendy Leisenring,<sup>2,4</sup> Veronique Erard,<sup>1,4</sup> Sachiko Seo,<sup>1,4</sup> Louise Kimball,<sup>1</sup> Lawrence Corey,<sup>1,3,5</sup> Steven A Pergam,<sup>1,2,3</sup> and Keith R. Jerome<sup>1,5</sup>

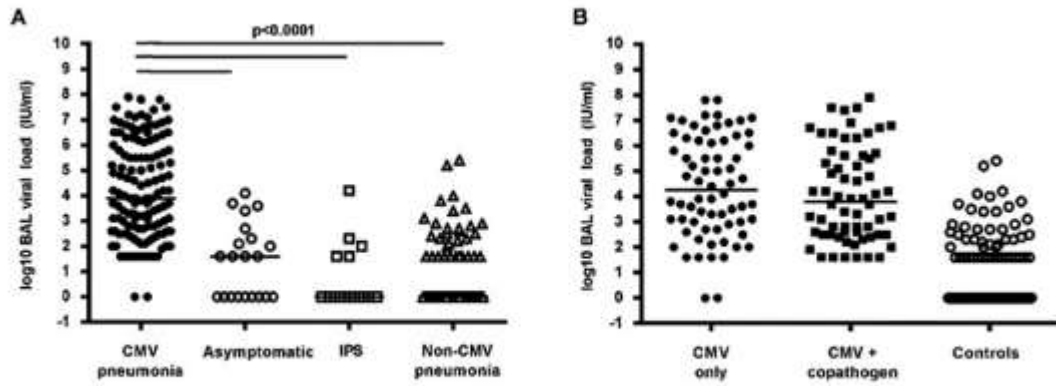
<sup>1</sup>Vaccine and Infectious Diseases Division and <sup>2</sup>Clinical Research Division, Fred Hutchinson Cancer Research Center, and <sup>3</sup>Department of Medicine, <sup>4</sup>Division of Biostatistics, and <sup>5</sup>Virology Division, Department of Laboratory Medicine, University of Washington, Seattle



**Figure 3.** Receiver operating characteristic (ROC) curves and predictive models. *A*, Patients with cytomegalovirus (CMV) pneumonia ( $n = 132$ ) versus controls with idiopathic pneumonia syndrome (IPS) or non-CMV pneumonia ( $n = 118$ ). The optimal cutoff was 99.7 IU/mL, with a sensitivity of 90.2% and specificity of 80.5%. *B*, Patients with CMV pneumonia ( $n = 132$ ) versus asymptomatic controls ( $n = 21$ ). The optimal cutoff was 203.3 IU/mL, with a sensitivity of 84.1% and a specificity of 76.2%.

The study tested bronchoalveolar lavage (BAL) fluid samples from 132 HCT recipients with CMV pneumonia and 139 controls by quantitative CMV and  $\beta$ -globin DNA-specific PCR.

CMV DNA load in BAL can be used to differentiate CMV pneumonia from pulmonary shedding



**Figure 1.** Quantitative cytomegalovirus (CMV) load in bronchoalveolar lavage (BAL) fluid. *A*, Viral load in BAL fluid was significantly higher in CMV pneumonia cases than in any of the control subgroups. *B*, Viral load in BAL fluid from CMV pneumonia cases did not differ according to the absence (CMV only) or presence of a copathogen (BAL spec-

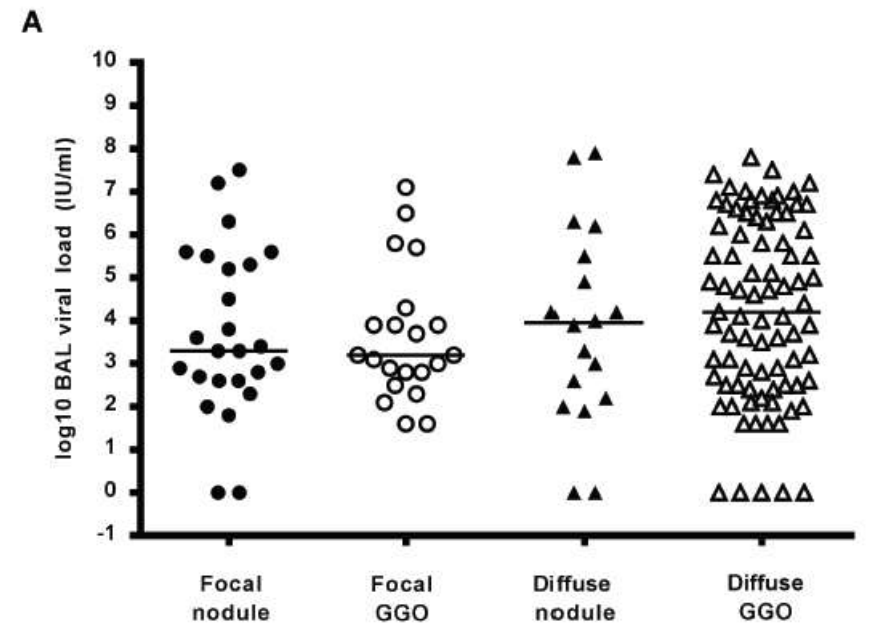
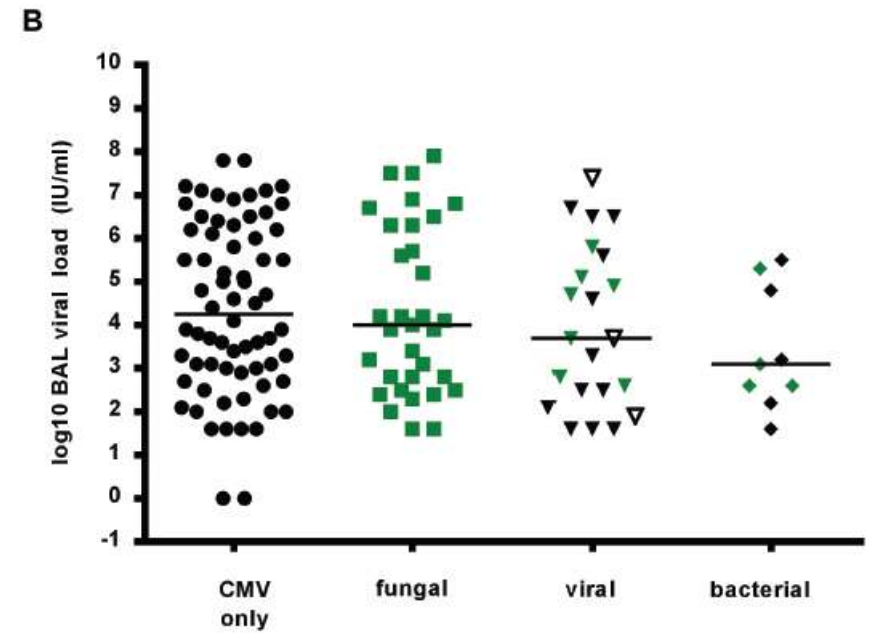
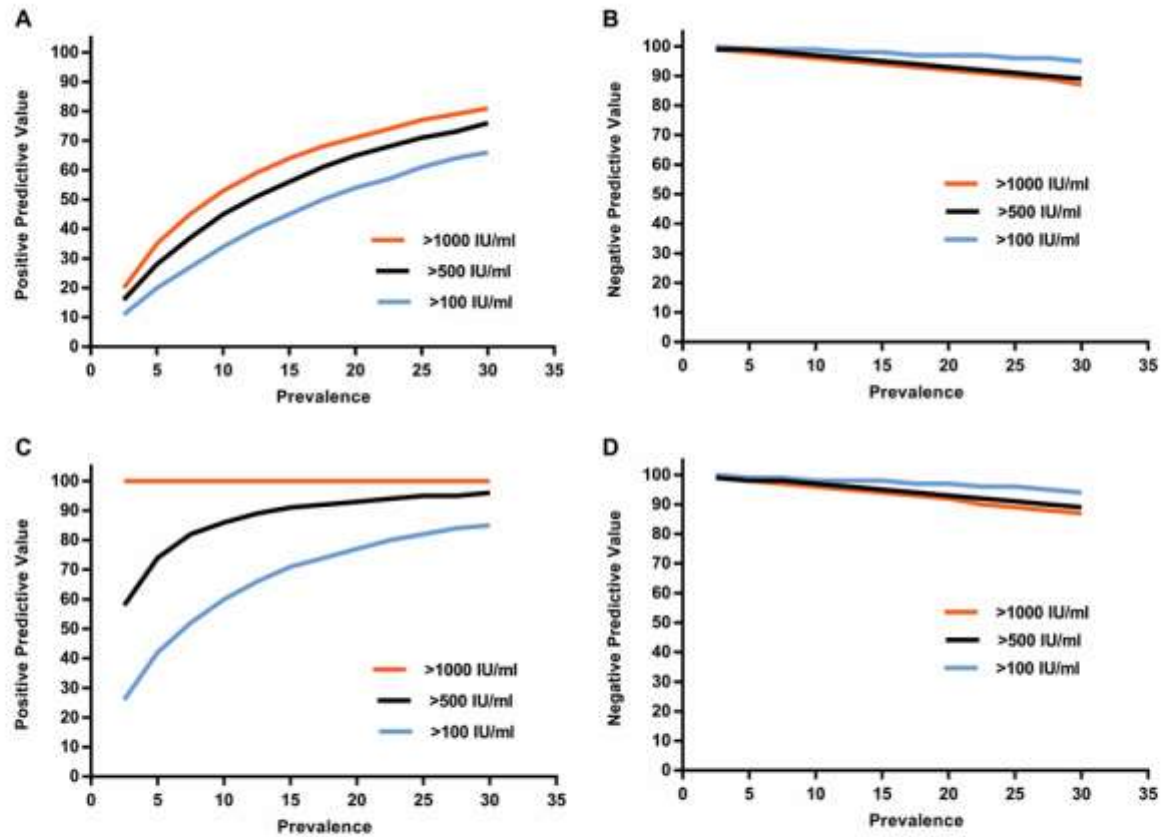


Figure 2. Quantitative cytomegalovirus (CMV) load in bronchoalveolar lavage (BAL) fluid according to possible effect modifiers. *A*, Viral load in BAL fluid from CMV pneumonia cases did not differ with respect to the radiologic appearance of the lungs at the time of the BAL.





Predictive models. Positive predictive values with thresholds of 100, 500, 1000 IU/mL across a range of cytomegalovirus (CMV) pneumonia prevalences in patients who underwent a bronchoalveolar lavage for evaluation of pulmonary infiltrates. *A*, Data for all patients (132 patients with CMV pneumonia and 118 controls with non-CMV pneumonia). *C*, Patients without antiviral treatment (99 patients with CMV pneumonia and 78 controls with non-CMV pneumonia). Corresponding negative predictive values are shown in panels *B* and *D*.

They recommend 500 IU/mL as a pragmatic cutoff in BAL because, although ROC analysis suggests lower cutoffs ( $\approx 200$  IU/mL)

Clinical Research Paper

# Diagnosis of cytomegalovirus pneumonia by quantitative polymerase chain reaction using bronchial washing fluid from patients with hematologic malignancies

Hwa Young Lee<sup>1,\*</sup>, Chin Kook Rhee<sup>1,\*</sup>, Joon Young Choi<sup>1</sup>, Hea Yon Lee<sup>1</sup>, Jong Wook Lee<sup>2,3</sup> and Dong Gun Lee<sup>3,4,5</sup>

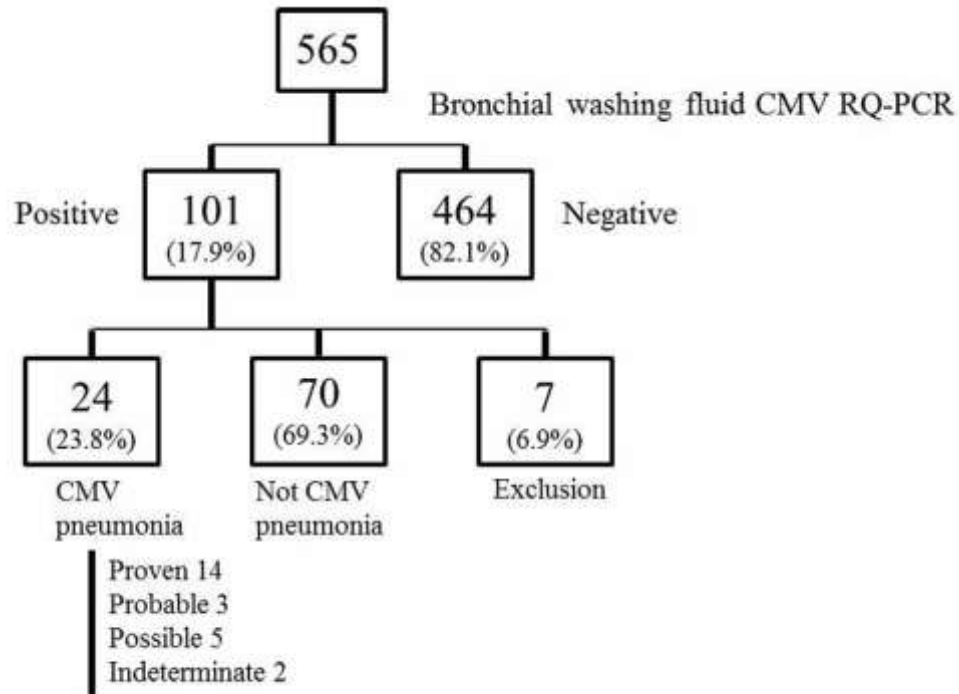


Figure 1: Flow chart of patients with hematologic malignancies who underwent bronchoscopy during the study period.

Median viral load in bronchial washing was much higher in pneumonia patients:  $\sim 1.8 \times 10^5$  copies/mL vs  $\sim 3 \times 10^3$  copies/mL in infection without pneumonia

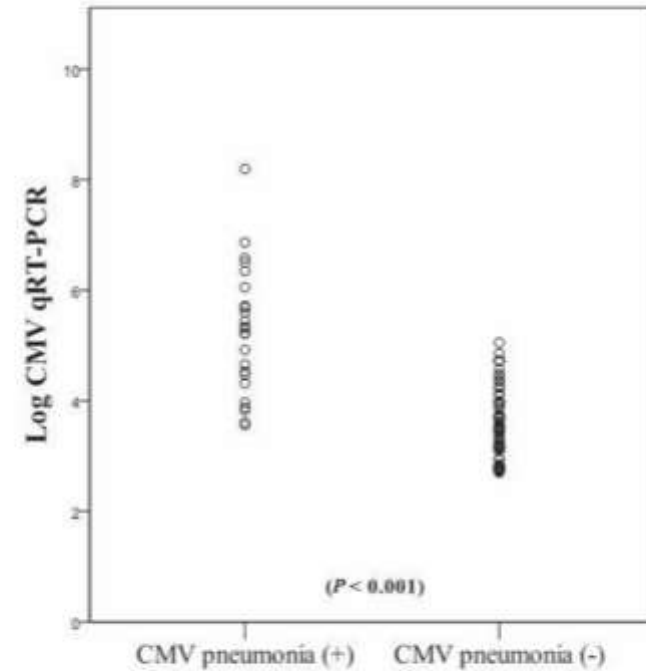
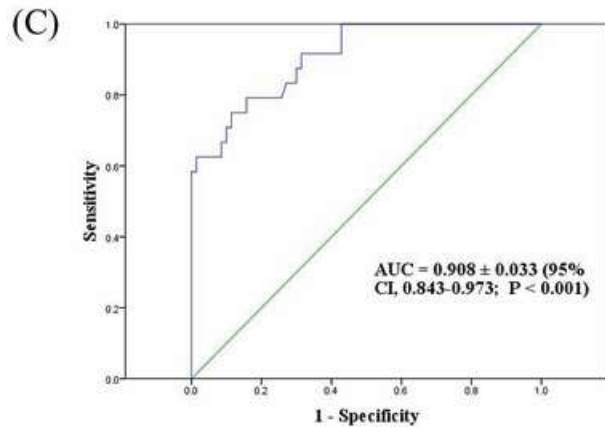
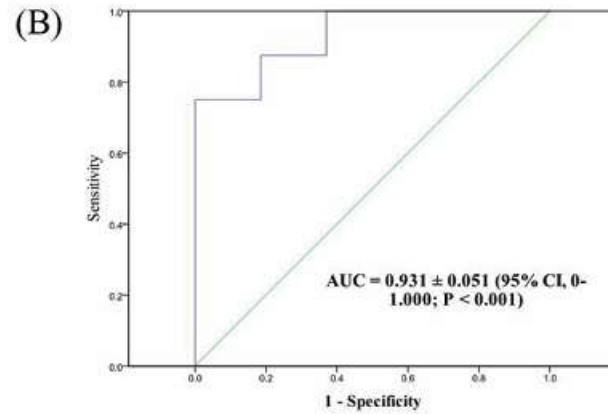
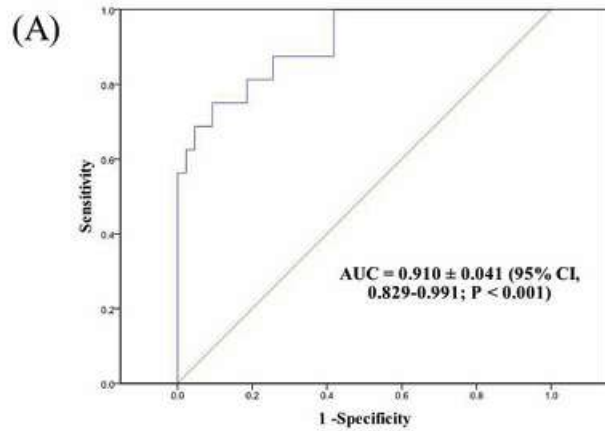


Figure 2: Distribution of CMV viral load in bronchial washing fluid. The median  $\log_{10}$  (CMV qRT-PCR copies/mL) values between the patients diagnosed CMV pneumonia and those who were not diagnosed CMV pneumonia were different significantly ( $P < 0.001$ ).



Different optimal cutoff values depending on whether the patient had a bone marrow transplant (BMT) or not Post-BMT:


**Receiver-operator characteristics (ROC) curve of patients who were diagnosed versus not diagnosed with CMV pneumonia but with a CMV load >380 copies/mL (2,470 IU/MI) by qRT-PCR. A. Post-bone marrow transplantation (BMT) patients; viral cut-off was 18,900 copies/mL B. no-BMT patients; viral cut-off was 316,415 copies/mL. C. All patients; viral cut-off was 28,774 copies/mL.**

**Comparison of quantitative polymerase chain reaction results between patients diagnosed with CMV pneumonia or not**

|  | <b>CMV pneumonia<br/>(n = 24)</b> | <b>Not CMV pneumonia<br/>(n = 70)</b> | <b><i>P</i></b>   |
|--|-----------------------------------|---------------------------------------|-------------------|
| <b>Bronchial washing fluid<br/>(copies/ml)</b> |                                   |                                       | <b>&lt; 0.001</b> |
| Mean   | 7,378,508.6                       | 10,899.2                              |                   |
| Median   | 187,224.5                         | 3,055                                 |                   |
| Minimum - Maximum                              | 3,642-156,666,945                 | 506-113,000                           |                   |
| <b>Blood (copies/ml)</b>                       |                                   |                                       | <b>0.006</b>      |
| Mean   | 683,659.1                         | 20,915.4                              |                   |
| Median   | 33,839.5                          | 5,486.5                               |                   |
| Minimum - Maximum                              | 882-5,570,000                     | 689-280,870                           |                   |

Both bronchial washing fluid and blood CMV viral loads are significantly elevated in patients diagnosed with CMV pneumonia.

# Cytomegalovirus (CMV) DNA quantification in bronchoalveolar lavage fluid of immunocompromised patients with CMV pneumonia

Elena Beam<sup>1</sup>  | Jeffrey J. Germer<sup>2</sup> | Brian Lahr<sup>3</sup> | Joseph D.C. Yao<sup>2</sup> | Andrew Harold Limper<sup>4</sup> | Matthew J. Binnicker<sup>2</sup> | Raymund R. Razonable<sup>1,5</sup>

The study analyzed CMV VL in BALF from 17 patients and 21 control subjects using an FDA-approved PCR assay calibrated to the WHO International Standard for CMV DNA

| Subject group | No. cases | CMV VL in BALF                          |  |
|---------------|-----------|---|--|
|               |           | Original results in IU/mL (median; IQR) | Normalized results in IU/10 <sup>6</sup> cells (median; IQR) |
| Controls      | 21        | 0; 0 to 0 <sup>a</sup>                  | 0; 0 to 0 <sup>a</sup>                                       |
| Cases (all)   | 17        | 656 000; 34 800 to >18 200 000          | 13.61; 3.03 to >160.00                                       |
| Possible      | 7         | 32 400; <274 to 130 400                 | 0.32; <0.01 to 5.57  |
| Probable      | 4         | 1 305 000; 742 000 to 9 780 000         | 67.35; 19.23 to 130.50                                       |
| Proven        | 6         | >18 200 000; 676 000 to >18 200 000     | >160.00; 13.61 to > 160.00                                   |

IQR, interquartile range; BALF, bronchoalveolar lavage fluid; VL, viral load.

<sup>a</sup>Three control subjects had detectable CMV VL in BALF, but results were all below the IQR (<25th percentile).

Normalizing to cell count allowed better standardization and showed a strong distinction: values >160 IU/10<sup>6</sup> cells were only seen in proven cases. This supports BAL CMV DNA quantification, especially normalized values, as a helpful tool in diagnosing CMV pneumonia

**TABLE 5** Sensitivity and specificity analyses of various viral load thresholds for diagnosing cytomegalovirus (CMV) pneumonia in BALF

| CMV pneumonia cases <sup>a,b</sup> | No. cases | Original results (IU/mL) |                   |                 |                 | Normalized results (IU/10 <sup>6</sup> cells) |                    |                 |                 |
|------------------------------------|-----------|--------------------------|-------------------|-----------------|-----------------|---|--------------------|-----------------|-----------------|
|                                    |           | AUC                      | VL threshold      | Sensitivity (%) | Specificity (%) | AUC   | VL threshold       | Sensitivity (%) | Specificity (%) |
| Proven, probable, and possible     | 17        | 0.979                    | ~274 <sup>b</sup> | 100.0           | 85.7            | 0.973   | ~0.01 <sup>b</sup> | 100.0           | 85.7            |
| Proven and probable                | 10        | 1.000                    | 234 000           | 100.0           | 100.0           | 1.000   | 4.76               | 100.0           | 100.0           |
| Proven                             | 6         | 1.000                    | 656 000           | 100.0           | 100.0           | 1.000   | 7.05               | 100.0           | 100.0           |
| Transplant-only                    | 12        | 0.990                    | 34 800            | 91.7            | 100.00          | 0.984   | 0.32               | 91.7            | 90.5            |

AUC, area under the curve; BALF, bronchoalveolar lavage fluid; VL, viral load.

<sup>a</sup>For each comparison, cases were compared to a total of 21 controls.

<sup>b</sup>CMV VL threshold estimates based on an assigned value when original VL was below the lower limit of quantification for BALF (ie, assume “positivity” for both original or normalized result when the raw VL is quantifiable).

Thresholds like 234,000 IU/mL or 4.76 IU/10<sup>6</sup> cells distinguished proven/probable CMV pneumonia from controls, with 100% sensitivity and specificity

## A Prospective Study of Plasma and Bronchoalveolar Lavage Fluid CMV DNA Load Quantification for the Diagnosis and Outcome of CMV Pneumonitis in Immunocompromised Hosts

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Jackrapong Bruminhent<sup>e,f</sup>

### Accuracy of cut-off values for plasma and BALF CMV DNA load in diagnosis of CMV pneumonitis

| CMV DNA load (IU/mL)                          | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|-----------------|-----------------|---------|---------|--------------|
| Cut-off value for plasma CMV DNA load (IU/mL) |                 |                 |         |         |              |
| 467   | 100             | 91.2            | 78.5    | 100     | 93.3         |
| 831   | 100             | 94.1            | 84.5    | 100     | 95.5         |
| 1,364   | 90.9            | 94.1            | 83.3    | 96.8    | 93.3         |
| Cut-off value for BALF CMV DNA load (IU/mL)   |                 |                 |         |         |              |
| 10,263  | 100             | 94.1            | 100     | 95.2    | 96.2         |
| 24,565  | 100             | 91.1            | 91.6    | 100     | 97.9         |
| 40,174  | 90.9            | 97.1            | 90.8    | 97.0    | 95.5         |

A prospective study - between October 2019 and October 2020, enrolled IC patients with suspected pneumonia.

Plasma and BALF samples were collected and quantified for CMV DNA using a RealTime CMV assay

# ROC

Positive correlation with an  $R^2$  value of 0.887 ( $p < 0.001$ ) between plasma CMV DNA loads and BALF CMV DNA loads.

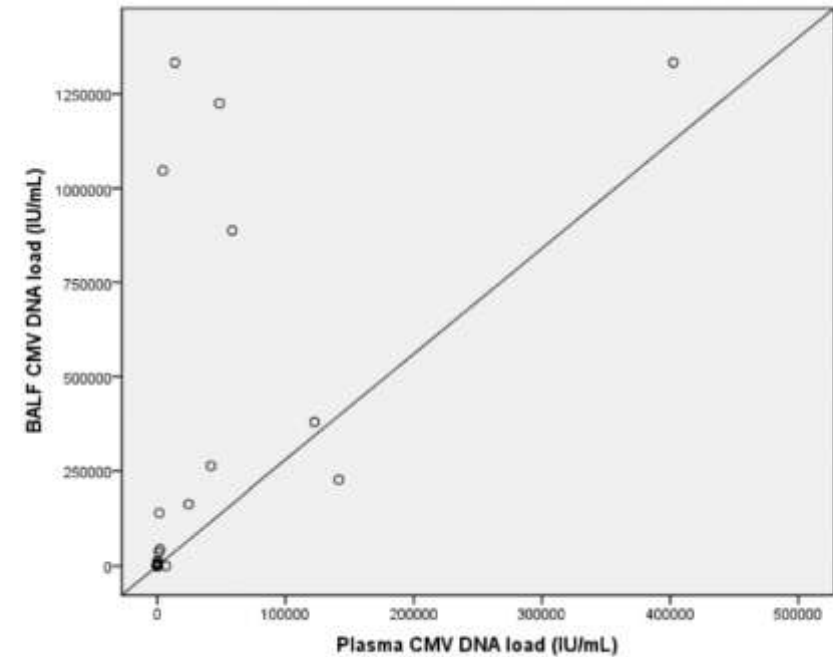


Figure 2. Correlation between plasma and bronchoalveolar lavage fluid (BALF) CMV DNA loads in immunocompromised patients ( $n=45$ ). Abbreviations: CMV, cytomegalovirus; BALF, bronchoalveolar lavage fluid.

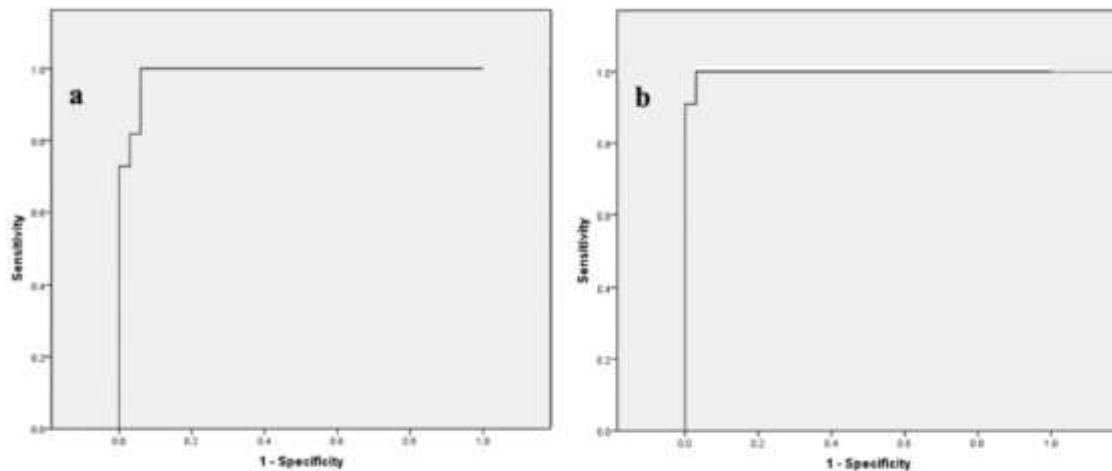


Figure 3. Receiver operating characteristic curve for (a) plasma CMV DNA load in the diagnosis of proven and/or probable CMV pneumonitis. The area under the curve = 0.9987 ( $\pm 0.013$ ) (b) BALF CMV DNA load in the diagnosis of proven and/or probable CMV pneumonitis ( $n=11$ ). The area under the curve is 0.997 ( $\pm 0.004$ )

Good diagnostic accuracy :

Plasma CMV DNA load at a cut-off of 831 IU/mL with an AUC of 0.9987, achieving 100% sensitivity and 94.1% specificity.

BALF CMV DNA load at a cut-off of 24,565 IU/mL showed an AUC of 0.997 with 100% sensitivity and 91.1% specificity

# CMV ANTIGENEMIA

- Detects CMV pp65 antigen inside peripheral blood leukocytes.
- Uses immunostaining with CMV-specific monoclonal antibodies.

#### Result & Interpretation:

- Positive: pp65-positive leukocytes detected → indicates active viral replication.
- The number of pp65-positive cells (reported as cells per 200,000 WBCs) is semi-quantitative.
- Quantitative: Number of positive cells correlates with viral load and disease risk.

# Diagnostic test accuracy of antigenaemia assay for PCR-proven cytomegalovirus infection—systematic review and meta-analysis

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## Summary of diagnostic test accuracy

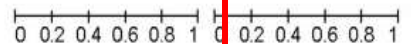
|              | All                       | SOT                       | HSCT                      | CNAkit                    | Brite Turbo              | Clonab                    |
|--------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|---------------------------|
| Studies      | 75                        | 26                        | 18                        | 14                        | 7                        | 15                        |
| PCR positive | 9058                      | 5123                      | 1580                      | 1218                      | 3335                     | 1082                      |
| PCR negative | 22 232                    | 10 219                    | 3635                      | 3025                      | 6720                     | 2660                      |
| DOR          | 30 (24–38)<br>$I^2 = 28%$ | 28 (18–42)<br>$I^2 = 34%$ | 25 (15–40)<br>$I^2 = 37%$ | 38 (22–64)<br>$I^2 = 22%$ | 35 (30–41)<br>$I^2 = 0%$ | 24 (13–44)<br>$I^2 = 32%$ |
| AUC          | 0.86 (0.83–0.88)          | 0.86 (0.81–0.90)          | 0.84 (0.80–0.88)          | 0.88 (0.99–0.92)          | 0.83 (0.78–0.88)         | 0.84 (0.78–0.90)          |
| Sensitivity  | 0.65 (0.59–0.70)          | 0.65 (0.56–0.73)          | 0.60 (0.49–0.70)          | 0.65 (0.55–0.75)          | 0.54 (0.34–0.73)         | 0.64 (0.52–0.75)          |
| Specificity  | 0.94 (0.93–0.95)          | 0.93 (0.91–0.95)          | 0.94 (0.91–0.97)          | 0.95 (0.91–0.97)          | 0.97 (0.94–0.98)         | 0.94 (0.89–0.96)          |
| PLR          | 10.9 (8.5–14.0)           | 9.7 (6.7–14.0)            | 10.3 (5.3–20.1)           | 11.9 (6.9–20.6)           | 16.4 (7.4–33.8)          | 9.9 (5.6–17.3)            |
| NLR          | 0.38 (0.32–0.44)          | 0.38 (0.29–0.47)          | 0.43 (0.32–0.55)          | 0.37 (0.27–0.48)          | 0.47 (0.28–0.68)         | 0.38 (0.27–0.52)          |

Abbreviations: PCR positive/negative, a number of subjects allowing repeated evaluation of same subjects; DOR, diagnostic odds ratio; AUC, area under hierarchical summary receiver operating characteristics curve; PLR, Positive likelihood ratio; NLR, Negative likelihood ratio; SOT, solid organ transplantation recipient; HSCT, haematopoietic stem cell transplantation recipient; CNAkit, CINAKit (Argene Biosoft); Brite Turbo, CMV Brite Turbo Kit (IQ Products); Clonab, Clonab CMV (Biotest).

The CMV antigenaemia assay shows good specificity and moderate sensitivity regardless of population or assay kit used.

| Study              | TP  | FP | FN  | TN   | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|--------------------|-----|----|-----|------|----------------------|----------------------|----------------------|----------------------|
| Alice 2006         | 239 | 4  | 77  | 55   | 0.76 [0.71, 0.80]    | 0.93 [0.84, 0.98]    |                      |                      |
| Ashokkumar 2015    | 7   | 1  | 1   | 30   | 0.88 [0.47, 1.00]    | 0.97 [0.83, 1.00]    |                      |                      |
| Beckmann 2011      | 99  | 14 | 145 | 462  | 0.41 [0.34, 0.47]    | 0.97 [0.95, 0.98]    |                      |                      |
| Bergallo 2008      | 22  | 2  | 2   | 3    | 0.92 [0.73, 0.99]    | 0.60 [0.15, 0.95]    |                      |                      |
| Boaretti 2013      | 47  | 0  | 56  | 163  | 0.46 [0.36, 0.56]    | 1.00 [0.98, 1.00]    |                      |                      |
| Boeckh 1997        | 56  | 6  | 46  | 220  | 0.55 [0.45, 0.65]    | 0.97 [0.94, 0.99]    |                      |                      |
| Boeckh 2004        | 122 | 29 | 122 | 1629 | 0.50 [0.44, 0.56]    | 0.98 [0.97, 0.99]    |                      |                      |
| Boivin 1998        | 26  | 1  | 21  | 124  | 0.55 [0.40, 0.70]    | 0.99 [0.96, 1.00]    |                      |                      |
| Boland 1992        | 36  | 9  | 54  | 102  | 0.40 [0.30, 0.51]    | 0.92 [0.85, 0.96]    |                      |                      |
| Bonon 2006         | 13  | 5  | 13  | 15   | 0.50 [0.30, 0.70]    | 0.75 [0.51, 0.91]    |                      |                      |
| Breda 2013         | 10  | 1  | 52  | 133  | 0.16 [0.08, 0.28]    | 0.99 [0.96, 1.00]    |                      |                      |
| Camargo 2001       | 61  | 10 | 44  | 47   | 0.58 [0.48, 0.68]    | 0.82 [0.70, 0.91]    |                      |                      |
| Cardenoso 2013     | 169 | 1  | 201 | 6    | 0.46 [0.41, 0.51]    | 0.86 [0.42, 1.00]    |                      |                      |
| Cathomas 1994      | 49  | 8  | 13  | 115  | 0.79 [0.67, 0.88]    | 0.93 [0.88, 0.97]    |                      |                      |
| Choi 2009          | 66  | 77 | 18  | 394  | 0.79 [0.68, 0.87]    | 0.84 [0.80, 0.87]    |                      |                      |
| Davila 2005        | 12  | 11 | 11  | 130  | 0.52 [0.31, 0.73]    | 0.92 [0.86, 0.96]    |                      |                      |
| Deback 2007        | 45  | 52 | 3   | 92   | 0.94 [0.83, 0.99]    | 0.64 [0.55, 0.72]    |                      |                      |
| Eckart 1996        | 4   | 0  | 0   | 1    | 1.00 [0.40, 1.00]    | 1.00 [0.03, 1.00]    |                      |                      |
| Flexman 2001       | 109 | 0  | 136 | 224  | 0.44 [0.38, 0.51]    | 1.00 [0.98, 1.00]    |                      |                      |
| Garrigue 2006      | 60  | 7  | 45  | 53   | 0.57 [0.47, 0.67]    | 0.88 [0.77, 0.95]    |                      |                      |
| Guiver 2001        | 128 | 24 | 22  | 188  | 0.85 [0.79, 0.91]    | 0.89 [0.84, 0.93]    |                      |                      |
| Hadaya 2003        | 124 | 0  | 91  | 106  | 0.58 [0.51, 0.64]    | 1.00 [0.97, 1.00]    |                      |                      |
| Halfon 2011        | 30  | 2  | 23  | 189  | 0.57 [0.42, 0.70]    | 0.99 [0.96, 1.00]    |                      |                      |
| Hebart 1996        | 47  | 1  | 4   | 57   | 0.92 [0.81, 0.98]    | 0.98 [0.91, 1.00]    |                      |                      |
| Hoe 2007           | 10  | 6  | 46  | 281  | 0.18 [0.09, 0.30]    | 0.98 [0.96, 0.99]    |                      |                      |
| Jullet 2010        | 16  | 0  | 4   | 12   | 0.80 [0.56, 0.94]    | 1.00 [0.74, 1.00]    |                      |                      |
| Khansarinejad 2015 | 69  | 20 | 110 | 980  | 0.39 [0.31, 0.46]    | 0.98 [0.97, 0.99]    |                      |                      |
| Kidd 1993          | 2   | 1  | 73  | 219  | 0.03 [0.00, 0.09]    | 1.00 [0.97, 1.00]    |                      |                      |
| Ksouri 2006        | 16  | 0  | 10  | 100  | 0.62 [0.41, 0.80]    | 1.00 [0.96, 1.00]    |                      |                      |
| Landini 1995       | 52  | 0  | 25  | 27   | 0.68 [0.56, 0.78]    | 1.00 [0.87, 1.00]    |                      |                      |
| Lazzarotto 1996    | 34  | 0  | 3   | 11   | 0.92 [0.78, 0.98]    | 1.00 [0.72, 1.00]    |                      |                      |
| Li 2003            | 43  | 6  | 18  | 231  | 0.70 [0.57, 0.81]    | 0.97 [0.95, 0.99]    |                      |                      |
| Lo 1997            | 37  | 2  | 17  | 52   | 0.69 [0.54, 0.80]    | 0.96 [0.87, 1.00]    |                      |                      |
| Lomas 1992         | 2   | 0  | 16  | 7    | 0.11 [0.01, 0.35]    | 1.00 [0.59, 1.00]    |                      |                      |
| Luchsinger 2015    | 34  | 15 | 7   | 123  | 0.83 [0.68, 0.93]    | 0.89 [0.83, 0.94]    |                      |                      |
| Madhavan 2007      | 60  | 0  | 4   | 6    | 0.94 [0.85, 0.98]    | 1.00 [0.54, 1.00]    |                      |                      |
| Mansy 1999         | 15  | 0  | 3   | 22   | 0.83 [0.59, 0.96]    | 1.00 [0.85, 1.00]    |                      |                      |
| Marenzi 1996       | 17  | 3  | 3   | 13   | 0.85 [0.62, 0.97]    | 0.81 [0.54, 0.96]    |                      |                      |
| Martiny 2011       | 42  | 6  | 39  | 129  | 0.52 [0.40, 0.63]    | 0.96 [0.91, 0.98]    |                      |                      |
| Masaoka 2001       | 22  | 33 | 1   | 50   | 0.96 [0.78, 1.00]    | 0.60 [0.49, 0.71]    |                      |                      |
| Matsunaga 1999     | 10  | 1  | 1   | 14   | 0.91 [0.59, 1.00]    | 0.93 [0.68, 1.00]    |                      |                      |
| Mauricio 2011      | 33  | 2  | 37  | 147  | 0.47 [0.35, 0.59]    | 0.99 [0.95, 1.00]    |                      |                      |
| Milan 2011         | 21  | 22 | 72  | 350  | 0.23 [0.15, 0.32]    | 0.94 [0.91, 0.96]    |                      |                      |
| Miller 2010        | 18  | 3  | 2   | 38   | 0.90 [0.68, 0.99]    | 0.93 [0.80, 0.98]    |                      |                      |

|                  |      |     |      |      |                   |                   |
|------------------|------|-----|------|------|-------------------|-------------------|
| Muller 2007      | 163  | 46  | 38   | 188  | 0.81 [0.75, 0.86] | 0.80 [0.75, 0.85] |
| Nitsche 2003     | 43   | 56  | 79   | 944  | 0.35 [0.27, 0.44] | 0.94 [0.93, 0.96] |
| Pang 2003        | 105  | 0   | 123  | 176  | 0.46 [0.39, 0.53] | 1.00 [0.98, 1.00] |
| Pellegrin 2000   | 49   | 33  | 4    | 197  | 0.92 [0.82, 0.98] | 0.86 [0.80, 0.90] |
| Peres 2010       | 11   | 2   | 10   | 7    | 0.52 [0.30, 0.74] | 0.78 [0.40, 0.97] |
| Piiparinen 2001  | 68   | 11  | 10   | 164  | 0.87 [0.78, 0.94] | 0.94 [0.89, 0.97] |
| Rautenberg 1999  | 23   | 8   | 25   | 13   | 0.48 [0.33, 0.63] | 0.62 [0.38, 0.82] |
| Rayas 2005       | 16   | 10  | 12   | 351  | 0.57 [0.37, 0.76] | 0.97 [0.95, 0.99] |
| Rhee 2011        | 92   | 20  | 156  | 631  | 0.37 [0.31, 0.43] | 0.97 [0.95, 0.98] |
| Sakamaki 1997    | 3    | 3   | 7    | 30   | 0.30 [0.07, 0.65] | 0.91 [0.76, 0.98] |
| Sanghavi 2008    | 203  | 258 | 18   | 2943 | 0.92 [0.87, 0.95] | 0.92 [0.91, 0.93] |
| Schirm 1999      | 129  | 10  | 29   | 112  | 0.82 [0.75, 0.87] | 0.92 [0.85, 0.96] |
| Schmidt 1994     | 11   | 2   | 4    | 79   | 0.73 [0.45, 0.92] | 0.98 [0.91, 1.00] |
| Schmidt 1995     | 47   | 4   | 11   | 202  | 0.81 [0.69, 0.90] | 0.98 [0.95, 0.99] |
| Schvoerer 2005   | 74   | 3   | 43   | 25   | 0.63 [0.54, 0.72] | 0.89 [0.72, 0.98] |
| Seehofer 2004    | 44   | 51  | 1    | 623  | 0.98 [0.88, 1.00] | 0.92 [0.90, 0.94] |
| Solano 2001      | 27   | 5   | 50   | 466  | 0.35 [0.25, 0.47] | 0.99 [0.98, 1.00] |
| Sowmya 2006      | 48   | 0   | 7    | 37   | 0.87 [0.76, 0.95] | 1.00 [0.91, 1.00] |
| Sun 2009         | 77   | 0   | 8    | 41   | 0.91 [0.82, 0.96] | 1.00 [0.91, 1.00] |
| Tantivanich 2002 | 8    | 3   | 3    | 86   | 0.73 [0.39, 0.94] | 0.97 [0.90, 0.99] |
| Thorne 2007      | 45   | 6   | 10   | 45   | 0.82 [0.69, 0.91] | 0.88 [0.76, 0.96] |
| Toulemonde 2000  | 139  | 69  | 26   | 209  | 0.84 [0.78, 0.89] | 0.75 [0.70, 0.80] |
| Velzing 1994     | 4    | 0   | 9    | 24   | 0.31 [0.09, 0.61] | 1.00 [0.86, 1.00] |
| Vlieger 1992     | 54   | 6   | 17   | 51   | 0.76 [0.64, 0.85] | 0.89 [0.78, 0.96] |
| Wang 2013        | 8    | 1   | 8    | 34   | 0.50 [0.25, 0.75] | 0.97 [0.85, 1.00] |
| Weber 1994       | 5    | 1   | 11   | 5    | 0.31 [0.11, 0.59] | 0.83 [0.36, 1.00] |
| Wie 1996         | 28   | 6   | 2    | 161  | 0.93 [0.78, 0.99] | 0.96 [0.92, 0.99] |
| Wrgart 1996      | 59   | 31  | 37   | 344  | 0.61 [0.51, 0.71] | 0.92 [0.88, 0.94] |
| Xue 2009         | 1428 | 180 | 1140 | 5172 | 0.56 [0.54, 0.58] | 0.97 [0.96, 0.97] |
| Yakushiji 2002   | 112  | 14  | 90   | 300  | 0.55 [0.48, 0.62] | 0.96 [0.93, 0.98] |



# CMV CULTURE

BAL

# Molecular and Culture-Based Bronchoalveolar Lavage Fluid Testing for the Diagnosis of Cytomegalovirus Pneumonitis

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## Clinical Diagnostic Criteria for CMV Pneumonitis

1. At least 2 of the following (a, b, c):
  - a. Signs/symptoms of pneumonia or evidence of organ dysfunction as manifested by 2 or more of the following:
    - i. Fever
    - ii. Cough
    - iii. Dyspnea or tachypnea (RR > 20)<sup>a</sup>
    - iv. FEV<sub>1</sub> ≤ 80% of baseline
    - v. New or increasing oxygen requirement
  - b. Host factors/predisposing conditions: SOT, HSCT, chemotherapy, rituximab, leukemia/lymphoma
  - c. Radiographic changes consistent with CMV pneumonia
    - i. CXR: reticulonodular or interstitial infiltrates
    - ii. CT: ground glass opacities (GGOs), small nodules (<1 cm), absence of large nodules, air space consolidation
- AND
2. If a lung biopsy or autopsy was performed, this showed no evidence of a more likely explanation (acute rejection, GVHD, BCNU toxicity).
- AND
3. Requires that no other pathogen is isolated in culture or identified by DFA/staining that represents a more likely cause of the patient's presentation and findings<sup>b</sup>.

Abbreviations: BCNU, bis-chloroethylnitrosourea; CMV, cytomegalovirus; CT, computed tomography; CXR, chest x-ray; DFA, direct fluorescent antibody; FEV<sub>1</sub>, forced expiratory volume in 1 second; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; HSV, herpes simplex virus; RR, respiratory rate; SOT, solid organ transplant.

<sup>a</sup> Although 20 breaths/minute is not a normal respiratory rate, this value was selected because it is frequently documented when the patient is breathing comfortably on room air.

<sup>b</sup> Coagulase-negative *Staphylococcus*, oropharyngeal flora, *Candida* spp, *Mycobacterium gordonae*, nontyped mycobacterial species, diphtheroids, HSV-1, HSV-2, *Enterococcus*, and *Penicillium* would not be considered pathogens.

### Test Characteristics of CMV Detection Methods in BAL Specimens

| Test                                | CMV Pneumonitis <sup>a</sup> |      | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-------------------------------------|------------------------------|------|-----------------|-----------------|---------|---------|
|                                     | (+)                          | (-)  |                 |                 |         |         |
| PCR (+)                             | 42                           | 56   | 91.3            | 94.6            | 42.9    | 99.6    |
| PCR (-)                             | 4                            | 972  |                 |                 |         |         |
| Shell vial culture (+)              | 25                           | 27   | 54.4            | 97.4            | 48.1    | 97.9    |
| Shell vial culture (-)              | 21                           | 1001 |                 |                 |         |         |
| Conventional culture (+)            | 13                           | 15   | 28.3            | 96.5            | 46.4    | 96.9    |
| Conventional culture (-)            | 33                           | 1013 |                 |                 |         |         |
| PCR and shell vial (+)              | 21                           | 23   | 45.7            | 97.8            | 47.4    | 97.6    |
| PCR and/or shell vial (-)           | 25                           | 1005 |                 |                 |         |         |
| PCR and culture (+)                 | 12                           | 13   | 26.1            | 98.7            | 48.0    | 96.8    |
| PCR and/or culture (-)              | 34                           | 1015 |                 |                 |         |         |
| Shell vial and culture (+)          | 10                           | 13   | 21.7            | 98.7            | 43.5    | 96.6    |
| Shell vial and/or culture (-)       | 36                           | 1015 |                 |                 |         |         |
| PCR, shell vial, and culture (+)    | 9                            | 12   | 19.6            | 98.8            | 42.6    | 96.6    |
| PCR, shell vial, and/or culture (-) | 37                           | 1016 |                 |                 |         |         |
| PCR and/or shell vial (+)           | 46                           | 60   | 100.0           | 94.2            | 43.4    | 100.0   |
| PCR and shell vial (-)              | 0                            | 968  |                 |                 |         |         |
| PCR and/or culture (+)              | 43                           | 58   | 93.4            | 94.4            | 42.6    | 99.7    |
| PCR and culture (-)                 | 3                            | 970  |                 |                 |         |         |
| Shell vial and/or culture (+)       | 28                           | 29   | 60.8            | 97.2            | 49.1    | 98.2    |
| Shell vial and culture (-)          | 18                           | 999  |                 |                 |         |         |
| PCR, shell vial, and/or culture (+) | 46                           | 61   | 100.0           | 94.1            | 42.3    | 100.0   |
| PCR, shell vial, and culture (-)    | 0                            | 967  |                 |                 |         |         |

Abbreviations: BAL, bronchoalveolar lavage; CMV, cytomegalovirus; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

<sup>a</sup> Criteria for CMV pneumonitis as defined in Table 1 and Methods.

PCR + culture together = good diagnostic yield

# Detection of cytomegalovirus in bronchoalveolar lavage fluid from immunocompromised patients with pneumonitis by viral culture and DNA quantification

C. Berengua<sup>a,b,e,\*1</sup>, E. Miró<sup>a,b,e</sup>, C. Gutiérrez<sup>b</sup>, M. Sánchez<sup>b</sup>, A. Mulero<sup>b</sup>, P. Ramos<sup>b</sup>, M. del Cuerpo<sup>b</sup>, A. Torrego<sup>c,e</sup>, I. García-Cadenas<sup>d,e</sup>, V. Pajares<sup>c,e</sup>, F. Navarro<sup>a,b,e</sup>, R. Martino<sup>d,e</sup>, N. Rabella<sup>a,b,e</sup>

A prospective study was conducted from January 2021 to July 2022, including 176 consecutive BAL samples from immunosuppressed patients with respiratory symptoms

HCMV culture and qPCR results obtained from BAL samples (PPV: positive predictive value; NPV: negative predictive value).

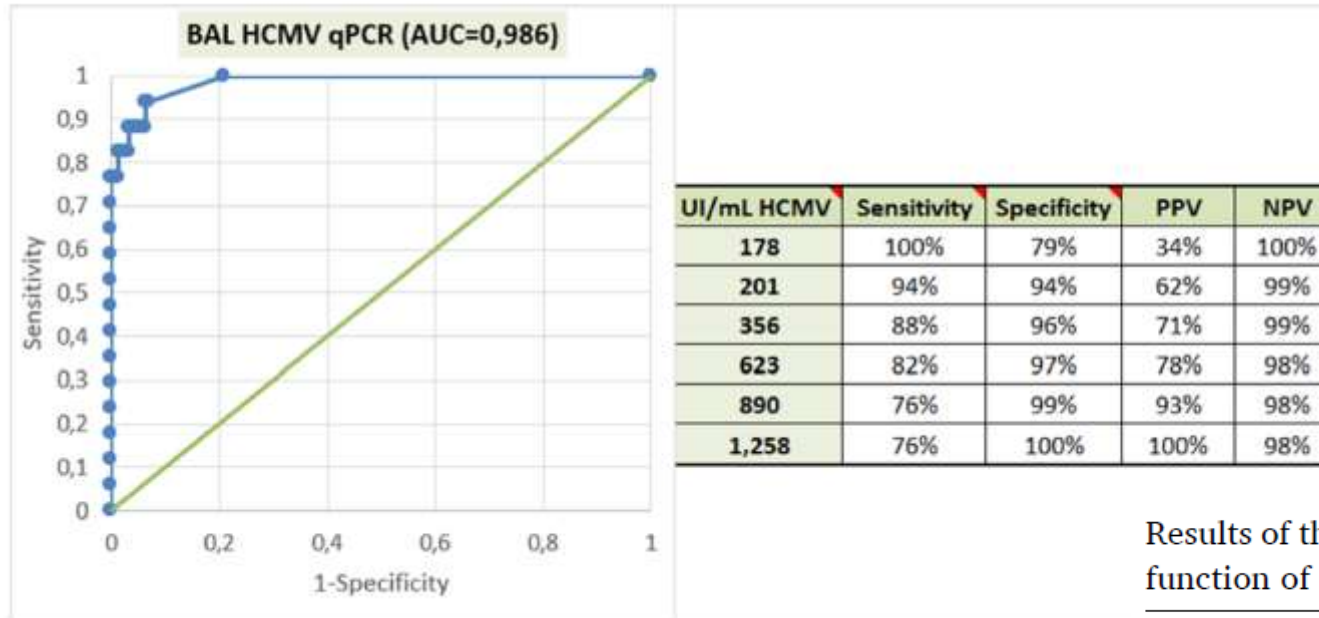
| n = 176       | Positive culture | Negative culture |          |
|---------------|------------------|------------------|----------|
| Positive qPCR | 17 (9.7%)        | 33 (18.7%)       | PPV 34%  |
| Negative qPCR | 0                | 126 (71.6%)      | NPV 100% |
| Total         | S 100%           | E 79.2%          |          |

Median viral load (VL) and qPCR cycle threshold (Ct) value in BAL of HCMV qPCR-positive samples according to the culture result.

|                                    | Positive culture (17)    | Negative culture (33) | p value   |
|------------------------------------|--------------------------|-----------------------|-----------|
| Median VL (Q1; Q3) BAL (IU/mL)     | 5038 (1008; 39,594)      | 178 (178; 248)        | p < 0.001 |
| Median VL (Q1; Q3) BAL (copies/mL) | 25,188 (5042; 197,974.5) | 890 (890; 1239)       | p < 0.001 |
| Median qPCR Ct value (Q1; Q3) BAL  | 32.10 (27.22; 34.11)     | 38.27 (36.11; 38.97)  | p < 0.001 |

higher viral loads (lower Ct) are associated with culture positivity and likely true infection.

ROC curve for BAL HCMV qPCR for diagnosis of HCMV pneumonia.  
Sensitivity, specificity, PPV and NPV values as a function of HCMV load in BAL.



ROC AUC = 0.986 → BAL CMV load is accurate.

Cutoff ~ >890–1250 IU/mL gives high specificity & PPV (true disease).

Concomitant DNAemia increases confidence in diagnosis.

Results of the HCMV DNAemia study and median HCMV viral load (IU/mL) as a function of BAL culture and qPCR result.

| DNAemia study                | BAL Positive culture and positive qPCR (17) | BAL Negative culture and positive qPCR (33) | BAL Negative culture and negative qPCR (126) |
|------------------------------|---|---|--|
| Not done (n)                 | 23.5% (4)                                   | 54.5% (18)                                  | 76.2% (96)                                   |
| Negative (n)                 | 0   | 3% (1)                                      | 15.9% (20)                                   |
| Positive (n)                 | 76.5% (13)                                  | 42.5% (14)                                  | 7.9% (10)                                    |
| • Median VL (Q1; Q3) (IU/mL) | • 4805 (461; 35,998)                        | • 222.5 (178; 982)                          | • 178 (178; 314.25)                          |

| Study                 | Verified Cutoff(s) and Method of PCR  | Specimen   | Reference standard  | Sensitivity / Specificity   |
|-----------------------|---|--|---|---|
| Dioverti et al., 2017 | <p><b>1700 IU/ml ( Plasma) in SOT</b><br/> <b>1350IU/ml ( plasma) in HSCT</b></p> <p>COBAS AmpliPrep/COBAS TaqMan CMV Test (CAP/CTM CMV),</p> | All patients were tested with a clinically approved quantitative nucleic acid test (QNAT) for CMV DNA, performed in plasma (PL). | Definitions of CMV infection and disease according to published guidelines, which included asymptomatic infection, CMV syndrome and end-organ disease | 1700IU/ml in plasma of SOT with sensitivity of 80% and 1350 IU/ml in plasma of HSCT with specificity of 74% and sensitivity of 87% to distinguish CMV disease from asymptomatic infection |
| Boeckh et al., 2017   | <p><b>500 IU/mL (BAL)</b></p> <p>Quantitative PCR targeting gB/pp65 genes</p>   | BAL fluid in HSCT patients   | <p>Shell vial assay</p> <p>Viral culture</p> <p>Direct fluorescent antibody testing (DFA)</p> <p>Cytology</p>   | Used ROC curves to distinguish CMV pneumonia vs shedding. Cutoff 500 IU/mL chosen as “reasonable with current prevalence” though threshold may vary.                                      |

| Study                       | Verified Cutoff(s) and Method of PCR                 | Specimen  | Reference standard   | Sensitivity / Specificity   |
|-----------------------------|--|---|--|---|
| Saksirisampant et al., 2022 | <p><b>831 IU/mL</b> (plasma)</p> <p>RT CMV assay</p> | <p>Plasma in mixed immunocompromised, BAL-PCR confirmed pneumonitis vs no pneumonitis</p> | <p>Diagnosis of CMV pneumonitis was classified as proven, probable, or possible as per guideline</p> | <p>AUC ~0.9987; sensitivity 100%, specificity 94.1%. BAL VL much higher in cases vs non-cases (median ~379,652 IU/mL vs ~0)</p>                     |
| Berengua et al., 2023       | <p><b>1,258 IU/mL</b> (BAL)</p> <p>ELITe MGB Kit</p> | <p>BAL in immunosuppressed with respiratory infection</p>                                 | <p>Viral culture of BAL fluid by both conventional and shell vial culture</p>                        | <p>Defines <math>\geq 1,258</math> IU/mL in BAL with sensitivity ~76%, specificity 100% to identify HCMV pneumonia vs culture and PCR criteria.</p> |

**IMAGING**

Georgeann McGuinness, MD • John V. Scholes, MD • Stuart M. Garay, MD  
 Barry S. Leitman, MD • Dorothy I. McCauley, MD • David P. Naidich, MD

**Cytomegalovirus Pneumonitis: Spectrum of Parenchymal CT Findings with Pathologic Correlation in 21 AIDS Patients<sup>1</sup>**

Table 2  
 CT Findings

| Patient | Ground-Glass Attenuation | Dense Consolidation | Masses              | Interstitial Reticulation | Miliary Nodules     | Bronchiectasis      | Bronchial Wall Thickening | Miscellaneous                |
|---------|--------------------------|---------------------|---------------------|---------------------------|---------------------|---------------------|---------------------------|------------------------------|
| 1       | ...                      | ...                 | Predominant finding | ...                       | ...                 | ...                 | ...                       | ...                          |
| 2       | ...                      | ...                 | Present             | Present                   | ...                 | Predominant finding | Predominant finding       | ...                          |
| 3       | ...                      | ...                 | Present             | Present                   | ...                 | Present             | Present                   | Cysts, cavities              |
| 4       | Present                  | ...                 | ...                 | Predominant finding       | ...                 | Present             | ...                       | Cysts, cavities              |
| 5       | Present                  | Present             | Present             | ...                       | ...                 | ...                 | ...                       | Adenopathy, pleural effusion |
| 6       | Predominant finding      | ...                 | Present             | Present                   | ...                 | ...                 | ...                       | ...                          |
| 7       | ...                      | ...                 | ...                 | ...                       | ...                 | Predominant finding | ...                       | Cysts, cavities              |
| 8       | Present                  | ...                 | ...                 | ...                       | ...                 | ...                 | Predominant finding       | Pleural effusion             |
| 9       | ...                      | ...                 | ...                 | ...                       | Predominant finding | ...                 | ...                       | Adenopathy                   |
| 10      | ...                      | Predominant finding | ...                 | ...                       | ...                 | ...                 | ...                       | ...                          |
| 11      | ...                      | Present             | Present             | ...                       | ...                 | ...                 | ...                       | Pleural effusion             |
| 12      | Predominant finding      | ...                 | Present             | ...                       | ...                 | ...                 | ...                       | ...                          |
| 13      | Predominant finding      | ...                 | ...                 | ...                       | ...                 | ...                 | ...                       | ...                          |
| 14      | ...                      | Present             | ...                 | ...                       | ...                 | ...                 | ...                       | Pleural effusion             |
| 15      | Present                  | Present             | ...                 | ...                       | ...                 | ...                 | ...                       | Adenopathy, pleural effusion |
| 16      | Predominant finding      | Present             | Present             | Present                   | ...                 | ...                 | ...                       | Adenopathy, pleural effusion |
| 17      | ...                      | ...                 | Predominant finding | ...                       | ...                 | ...                 | ...                       | ...                          |
| 18      | ...                      | Present             | Present             | Present                   | ...                 | ...                 | ...                       | Adenopathy, pleural effusion |
| 19      | Present                  | ...                 | Predominant finding | ...                       | ...                 | ...                 | ...                       | ...                          |
| 20      | ...                      | ...                 | Predominant finding | ...                       | ...                 | ...                 | ...                       | ...                          |
| 21      | ...                      | ...                 | ...                 | ...                       | ...                 | Predominant finding | Present                   | ...                          |

- 21 AIDS patients, with histologically confirmed CMV infection with no other pulmonary infections
- Retrospective review from 1991-1993
- Radiologic (CT) patterns: ground-glass, dense consolidation, nodules, and mass like opacities

# Thin-Section CT Findings in 32 Immunocompromised Patients with Cytomegalovirus Pneumonia Who Do Not Have AIDS

| TABLE I CT Findings of Bilateral Abnormalities |                                   |                        |                           |                           |   |                          |                     |
|--|-----------------------------------|------------------------|---------------------------|---------------------------|---|--------------------------|---------------------|
| Patient  | Ground-Glass Attenuation (n = 21) | Consolidation (n = 19) | Multiple Nodules (n = 19) | Nodules with Halo (n = 7) | Thickening of Bronchovascular Bundles (n = 7) | Pleural Effusion (n = 7) | Tree-in-Bud (n = 4) |
| 1  | —                                 | +++                    | +                         | +                         | —   | +                        | —                   |
| 2  | —                                 | +                      | —                         | —                         | —   | +                        | —                   |
| 3  | +                                 | —                      | +                         | —                         | —   | —                        | —                   |
| 4  | +++                               | +                      | +                         | +                         | +   | +                        | —                   |
| 5  | +                                 | —                      | —                         | —                         | —   | —                        | —                   |
| 6  | +++                               | +                      | —                         | —                         | +   | —                        | —                   |
| 7  | +                                 | +                      | —                         | —                         | —   | —                        | +                   |
| 8  | +++                               | +                      | —                         | —                         | +   | —                        | —                   |
| 9  | —                                 | +                      | +++                       | —                         | —   | —                        | —                   |
| 10   | —                                 | +                      | —                         | —                         | —   | +                        | —                   |
| 11   | +                                 | +                      | —                         | —                         | +   | —                        | +                   |
| 12   | —                                 | +++                    | —                         | —                         | —   | —                        | +                   |
| 13   | +++                               | —                      | +                         | —                         | —   | —                        | —                   |
| 14   | —                                 | —                      | +++                       | +                         | —   | —                        | —                   |
| 15   | +                                 | —                      | —                         | —                         | —   | —                        | —                   |
| 16   | —                                 | +++                    | —                         | —                         | —   | —                        | —                   |
| 17   | —                                 | —                      | +++                       | —                         | —   | —                        | —                   |
| 18   | +                                 | —                      | +++                       | —                         | —   | —                        | —                   |
| 19   | —                                 | —                      | +++                       | +                         | —   | —                        | —                   |
| 20   | +                                 | —                      | +++                       | +                         | —   | —                        | —                   |
| 21   | +                                 | +                      | +                         | —                         | +   | —                        | —                   |
| 22   | +                                 | +++                    | +                         | —                         | —   | —                        | —                   |
| 23   | —                                 | —                      | +                         | —                         | —   | —                        | —                   |
| 24   | +                                 | +                      | +++                       | +                         | —   | —                        | —                   |
| 25   | +++                               | —                      | +                         | —                         | +   | —                        | —                   |
| 26   | +++                               | —                      | +                         | —                         | —   | —                        | —                   |
| 27   | +                                 | —                      | +                         | —                         | +   | —                        | —                   |
| 28   | +                                 | +++                    | —                         | —                         | —   | +                        | +                   |
| 29   | —                                 | +++                    | —                         | —                         | —   | +                        | —                   |
| 30   | +++                               | +                      | —                         | —                         | —   | +                        | —                   |
| 31   | +++                               | +                      | +                         | —                         | —   | —                        | —                   |
| 32   | +++                               | +                      | +                         | +                         | —   | —                        | —                   |

Note.—Dash (—) = not present, +++ = predominant finding, + = present.

- Retrospective review of 32 IC patients without AIDS who had a histologically proven diagnosis of CMV pulmonary infection and had undergone CT
- Areas of ground-glass opacification were seen in 21 (66%) of 32 patients .
- Multiple nodules were identified in 19 patients (59%)

# CO-INFECTIONS

# Differences and similarities of cytomegalovirus and pneumocystis pneumonia in HIV-negative immunocompromised patients – thin section CT morphology in the early phase of the disease

<sup>1</sup>M N VOGEL, MD, <sup>1</sup>H BRODOEFEL, MD, <sup>2</sup>T HIERL, MD, <sup>3</sup>R BECK, MD, <sup>4</sup>W A BETHGE, MD, <sup>1</sup>C D CLAUSSEN, PROF and <sup>1</sup>M S HORGER, MD

- Both CMV and PcP pneumonia often show similar CT morphologies in the early disease phases, such as ground-glass opacities (GGO) and consolidations, making it difficult to distinguish between the two solely based on imaging

| Criteria                          | CMV pneumonia n=31 | PcP n=27 | Kappa | $\chi^2$ |
|-----------------------------------|--------------------|----------|-------|----------|
| GGO and consolidation             |                    |          |       |          |
| apical <sup>a</sup>               | 6 (19%)            | 19 (70%) | 0.8   | 16.02    |
| mosaic pattern <sup>a</sup>       | 3 (10%)            | 14 (54%) | 0.8   | 13.06    |
| unsharply demarcated <sup>b</sup> | 20 (65%)           | 6 (23%)  | 0.5   | 10.17    |
| GGO                               |                    |          |       |          |
| homogeneous <sup>b</sup>          | 5 (16%)            | 14 (54%) | 0.7   | 9.28     |
| Small nodules <sup>b</sup>        | 15 (48%)           | 3 (11%)  | 0.8   | 10.07    |

GGO, ground-glass opacity; PcP, Pneumocystis pneumonia; CMV pneumonia, cytomegalovirus pneumonia; Kappa, calculated correlation of both viewers, observations using Kappa-statistic;  $\chi^2$ , results of the statistical calculations using  $\chi^2$ -test.

PcP is more likely when:  
 GGO/consolidation have an apical distribution,  
 There is a mosaic pattern,  
 GGO is homogeneous.

CMV pneumonia is more likely when:  
 There are small nodules,  
 GGO and consolidation have unsharp (unclear) borders

Distribution of the CT morphological criteria with significant differentiating frequency. Values relate to a unanimous decision from both observers. The criteria are listed according to their significance

| Criteria                          | CMV pneumonia n=31 | PcP n=27 | Kappa | $\chi^2$ |
|-----------------------------------|--------------------|----------|-------|----------|
| GGO and consolidation             |                    |          |       |          |
| apical <sup>a</sup>               | 6 (19%)            | 19 (70%) | 0.8   | 16.02    |
| mosaic pattern <sup>a</sup>       | 3 (10%)            | 14 (54%) | 0.8   | 13.06    |
| unsharply demarcated <sup>b</sup> | 20 (65%)           | 6 (23%)  | 0.5   | 10.17    |
| GGO                               |                    |          |       |          |
| homogeneous <sup>b</sup>          | 5 (16%)            | 14 (54%) | 0.7   | 9.28     |
| Small nodules <sup>b</sup>        | 15 (48%)           | 3 (11%)  | 0.8   | 10.07    |

# Cytomegalovirus detection is associated with ICU admission in non-AIDS and AIDS patients with *Pneumocystis jirovecii* pneumonia

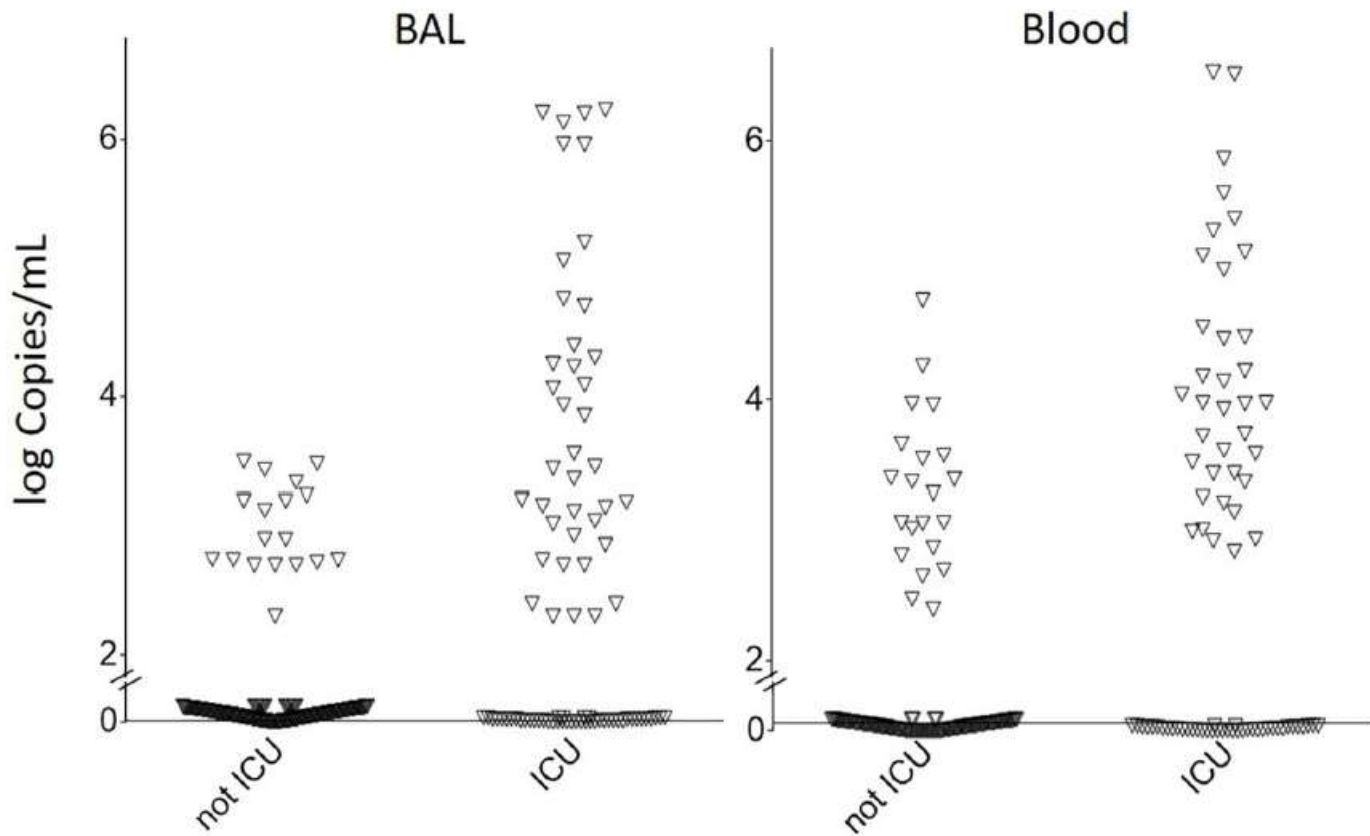
Alexandre Perret<sup>1,2</sup>, Marion Le Marechal<sup>1,2</sup>, Raphaelae Germe<sup>2,3</sup>, Daniele Maubon<sup>2,4</sup>, Cécile Garnaud<sup>2,4</sup>, Johan Noble<sup>5</sup>, Aude Boignard<sup>6</sup>, Loïc Falque<sup>7</sup>, Mathieu Meunier<sup>8</sup>, Théophile Gerster<sup>9</sup>, Olivier Epaulard<sup>1,2,10\*</sup>

Patient characteristics according to cytomegalovirus (CMV) detection in bronchoalveolar lavage (BAL) or blood.

|   | BAL CMV viral load<br>(N = 227)  |                    |        | Blood CMV viral load<br>(N = 194) |                    |        |
|---|--|--------------------|--------|-----------------------------------|--------------------|--------|
|   | <3 log*<br>(N = 190)   | ≥3 log<br>(N = 37) | p      | <3 log*<br>(N = 146)              | ≥3 log<br>(N = 48) | p      |
| Age (years)<br>Median [IQR]                                     | 65 [53–73]   | 66 [50–72]         | 0.673  | 62 [52–73]                        | 67 [57–73]         | 0.213  |
| Blood lymphocyte count (G/L)<br>Median [IQR]                    | High CMV viral load (≥3 log IU/mL) in BAL or blood is associated with more severe immunosuppression, coinfection (esp. <i>P. jirovecii</i> ), ICU admission, and prolonged ICU stay. |                    |        |                                   |                    | 0.185  |
| Gammaglobulin in blood (g/L)<br>Median [IQR]                    |  |                    |        |                                   |                    | 0.312  |
| <i>P. jirovecii</i> load in BAL (log copies/mL)<br>Median [IQR] | 4.0 [3.7–5.0]  | 5.7 [4.0–8.1]      | <0.001 | 4.7 [3.7–5.0]                     | 5.7 [4.4–8.1]      | <0.001 |
| ICU admission N (%)   | 54/190 (28.4%)   | 29/37 (78.4%)      | <0.001 | 43/146 (29.4%)                    | 33/48 (68.7%)      | <0.001 |
| ICU length of stay (days)                                       | 9 [4–18]   | 18 [9–31]          | 0.007  | 10 [4–14]                         | 19 [8–31]          | 0.001  |
| Mortality at 28 days N (%)                                      | 21/190 (11.0%)   | 5/37 (13.5%)       | 0.8814 | 17/146 (12.0%)                    | 4/48 (11.6%)       | 0.709  |

ICU = intensive care unit; BAL = bronchoalveolar lavage.

\* including patients with undetectable CMV viremia.



Bronchoalveolar lavage (BAL) and blood CMV viral load in patients with or without intensive care unit (ICU) admission.

Patients in ICU had much higher CMV loads (both BAL and blood) compared to non-ICU patients.

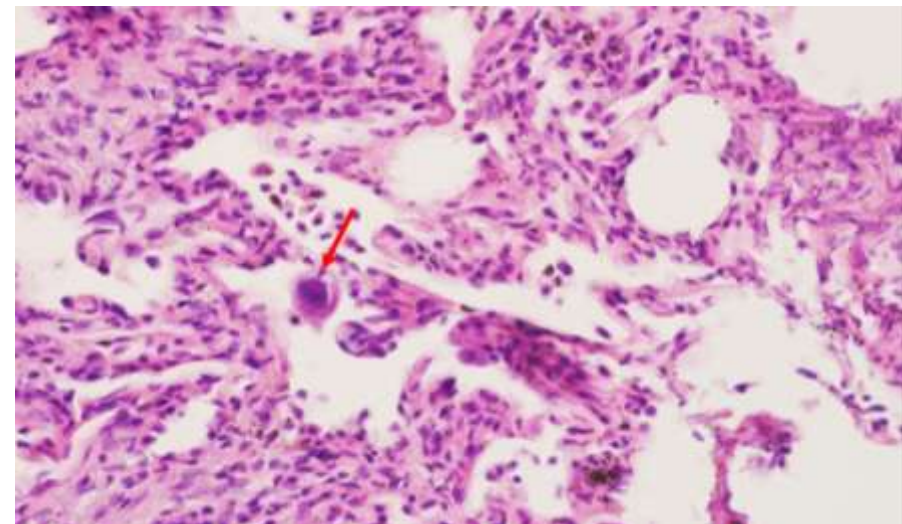
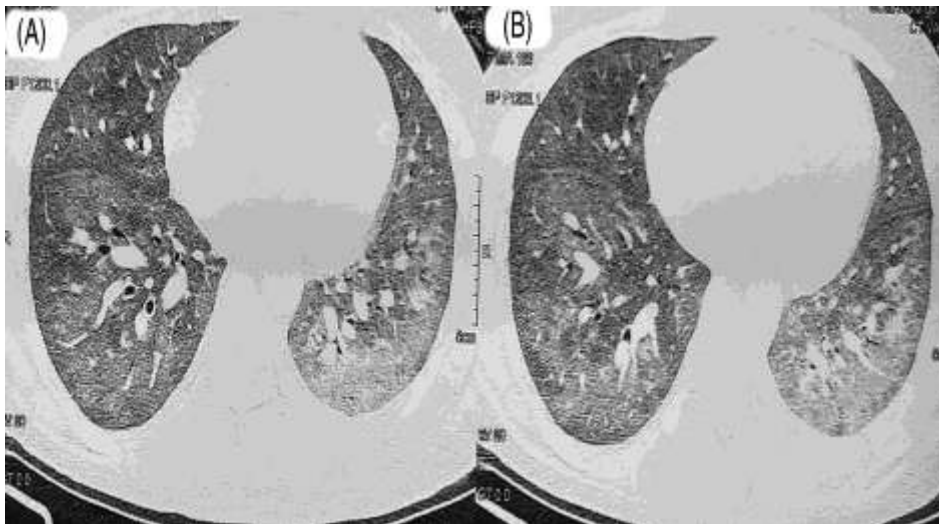
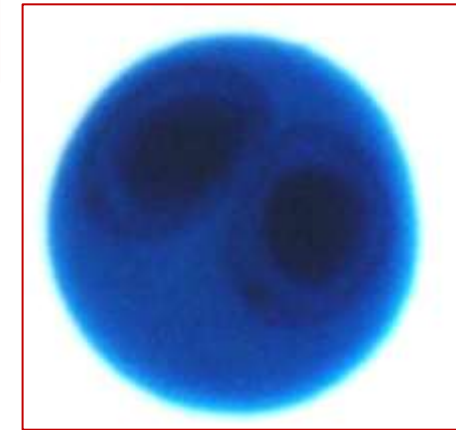
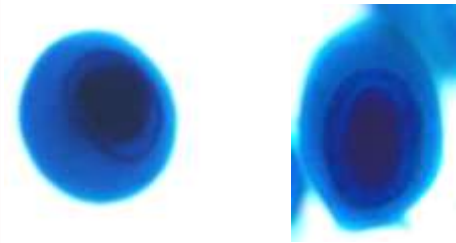
LAB INVESTIGATIONS  
AVAILABLE AT PGI?

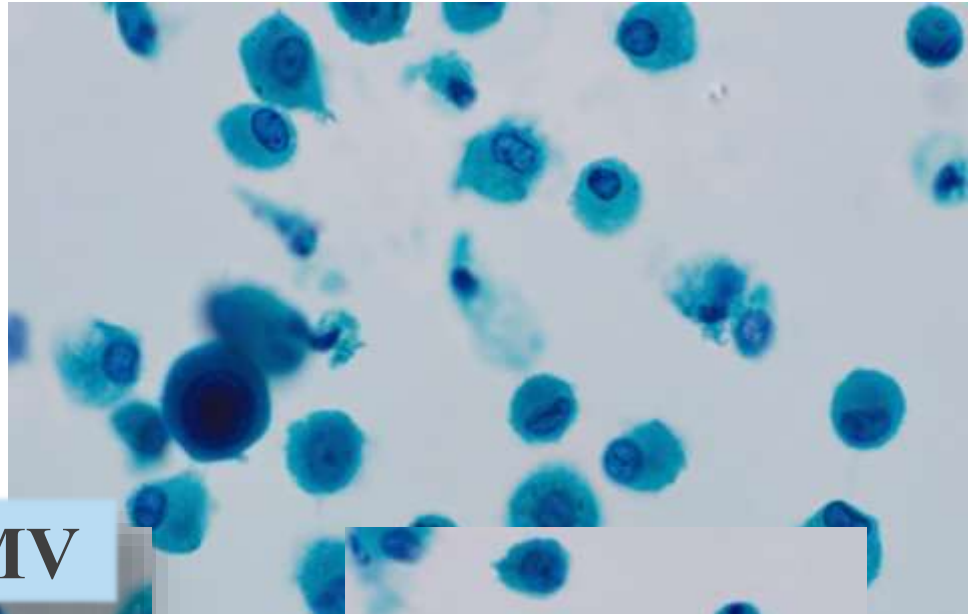
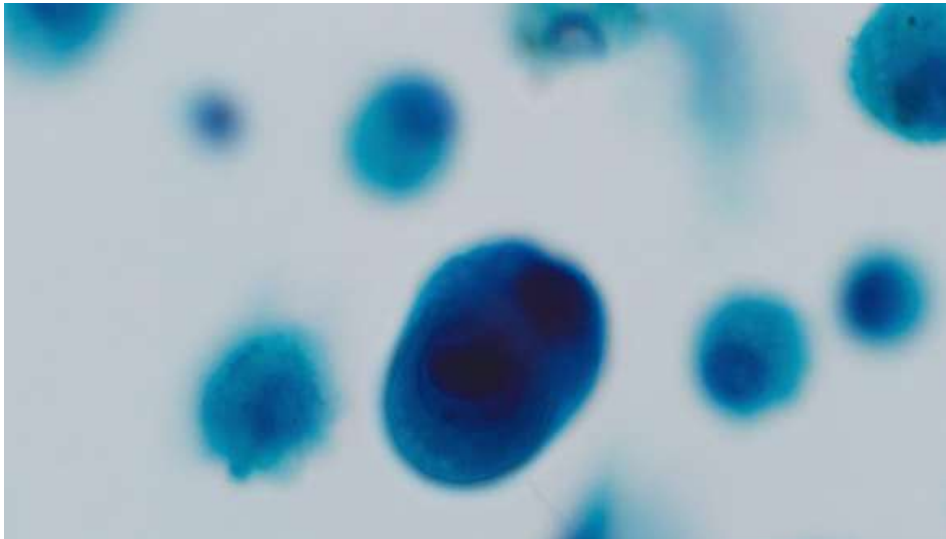
- Serum qualitative PCR – Nested PCR ( In house)
- Serum Quantitative PCR – commercial kit Tru CMV PCR ( real time PCR)
- BAL qualitative PCR – Nested PCR ( In house)
- BAL Serum Quantitative PCR , culture and PP65 – Not available

## The need for “eagle-eyed screening”: Owl's eyes in bronchoalveolar lavage cytology

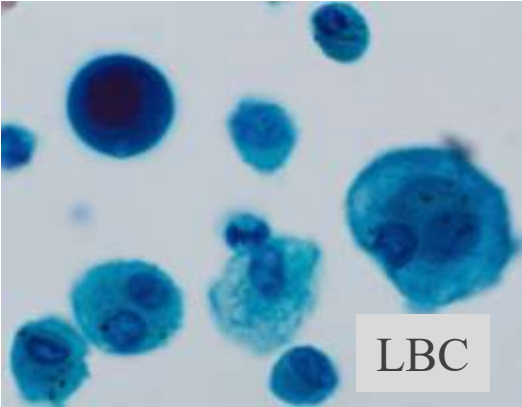
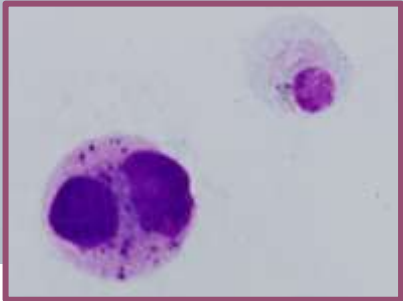
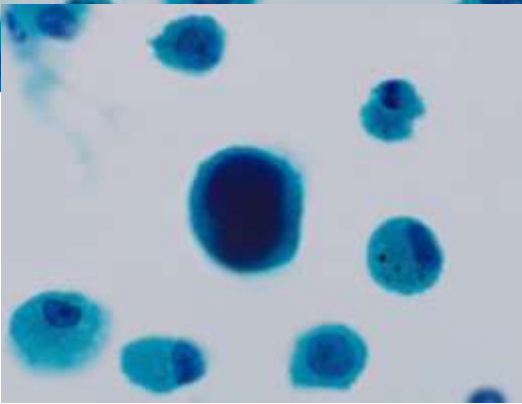
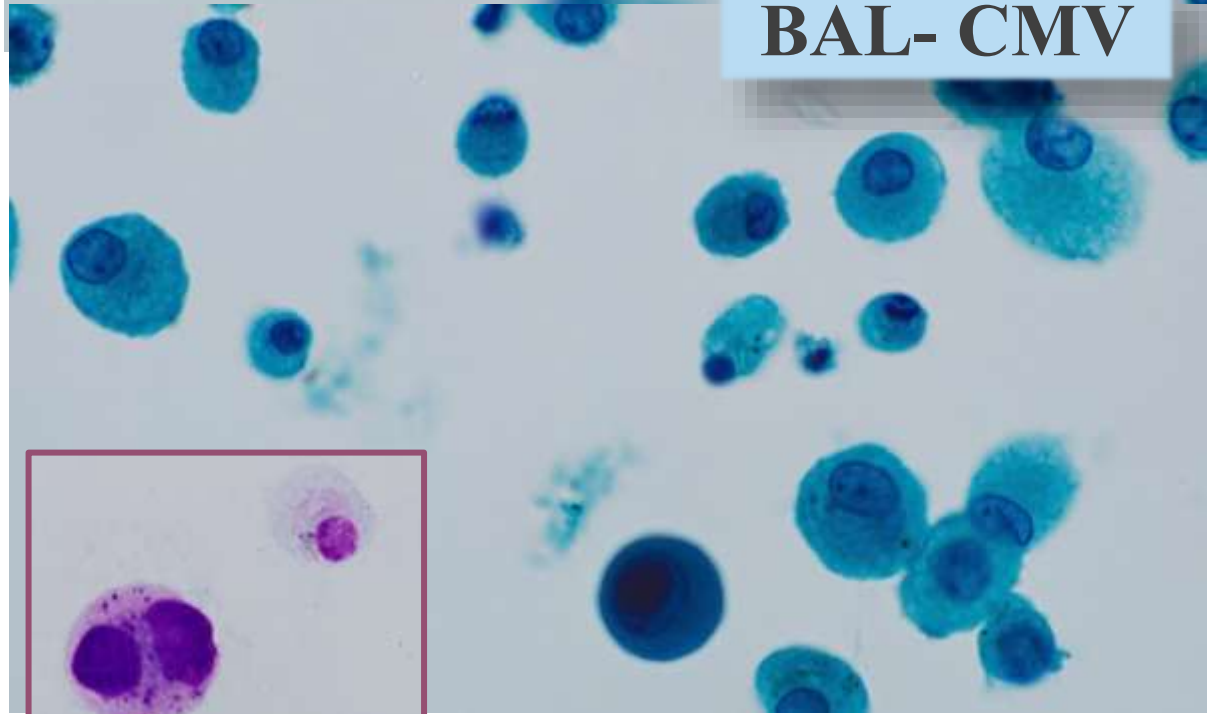
Nisha Duggal MD<sup>1</sup> | Parikshaa Gupta MD, DNB, MIAC<sup>1</sup> |  
Nivetha Ambalavanan MD<sup>2</sup> | Nalini Gupta MD, DNB, MIAC<sup>1</sup> | Valliappan Muthu MD<sup>3</sup>

- 36 M, HIV +ve
- Mason by occupation
- Presented with chronic cough, shortness of breath, and fever





**BAL- CMV**



LBC

# CASE 1

- 30yr male ,
- Dry cough for 2 months
- Breathlessness for 2 weeks
- Fever for 1 day 5 days back

## Lab Investigations :

- HIV - Reactive
- CD4 - 15(11/6/25)
- HCV/HBsAg :Non reactive
- BAL Genexpert(7/6/25) - Negative
- BAL Pneumocystis IFA(7/6/25) -Negative



Treated for PCP, as there was no improvement, treated for CMV pneumonitis

**Department of Virology**  
(Lab No. 606, Research Block A, level VI)  
PGIMER, Chandigarh

Name: **NARINDER SINGH** Age: **30** Gender: **Male**

Diagnosis: \_\_\_\_\_

CR No: **202502833947** OPDWard: **RICU-05** Lab No: **CM8-171**

Special Clinical No: \_\_\_\_\_

| Test Name                                   | Result in IU/ml                     |
|---|-------------------------------------|
| CMV Viral load:                             |                                     |
| Quantitative Real Time PCR in plasma sample | <b><math>5.2 \times 10^4</math></b> |

Real Time PCR kit Name: Tru PCR kit  
Nucleic Acid Extraction : Nuclisense Easymag extraction / Qiagen kit extraction

| Interpretation:                               |   |
|---|---|
| Result in copy number                         | Interpretation  |
| Target not detected                           | CMV DNA not detected.   |
| < 50 IU/ml                                    | CMV DNA detected less than 50 IU/ml. These results should be interpreted with caution.  |
| Between 50 IU/ml and $1.12 \times 10^8$ IU/ml | Calculated results greater than or equal to 50 CMV IU/ml and less than or equal to $1.12 \times 10^8$ IU/ml are within the linear range of the assay. |
| > $1.12 \times 10^8$ IU/ml                    | CMV DNA detected above the linear range of the assay.   |

Copies / ml = IU/ml x 2.15 (conversion factor)

Technician: \_\_\_\_\_ Senior Resident: **M. Sethi** Faculty: **Dr. Arbab Ghosh**  
Associate Professor  
Deptt. of Virology  
PGIMER, Chandigarh

At start of CMV treatment

**Department of Virology**  
(Lab No. 606, Research Block A, level VI)  
PGIMER, Chandigarh

Name: **NARINDER SINGH** Age: **30 Yr** Gender: **M**

Diagnosis: **PL HIV**

CR No: **202502833947** OPDWard: **Patna Med** Lab No: **CMQ 189**

Special Clinical No: \_\_\_\_\_

| Test Name                                   | Result in IU/ml                           |
|---|---|
| CMV Viral load:                             |   |
| Quantitative Real Time PCR in plasma sample | <b><math>5.7 \times 10^4</math> IU/ml</b> |

Real Time PCR kit Name: Tru PCR kit  
Nucleic Acid Extraction : Nuclisense Easymag extraction / Qiagen kit extraction

| Interpretation:                               |   |
|---|---|
| Result in copy number                         | Interpretation  |
| Target not detected                           | CMV DNA not detected.   |
| < 50 IU/ml                                    | CMV DNA detected less than 50 IU/ml. These results should be interpreted with caution.  |
| Between 50 IU/ml and $1.12 \times 10^8$ IU/ml | Calculated results greater than or equal to 50 CMV IU/ml and less than or equal to $1.12 \times 10^8$ IU/ml are within the linear range of the assay. |
| > $1.12 \times 10^8$ IU/ml                    | CMV DNA detected above the linear range of the assay.   |

Copies / ml = IU/ml x 2.15 (conversion factor)

Technician: \_\_\_\_\_ Senior Resident: \_\_\_\_\_ Faculty: **Dr. Arbab Ghosh**  
Associate Professor  
Deptt. of Virology  
PGIMER, Chandigarh

After 2 weeks of CMV treatment

# CASE 2

Similar symptoms with similar CT pattern

Treated for PCP, as there was improvement, not treated for CMV pneumonitis

**Department of Virology**  
(Lab No. 605, Phase III Block A, level VI)  
PGIMER, Chandigarh

Name: *Jai Bhagwan*    Age: *35 years*    Gender: *Male*

Diagnosis: *HIV(+), CD4 = 54, Pcp(+)*

CR No: *202502695412*    OPDW: *RICU*    Lab No: *CMQ-16*

Special Clinical No. \_\_\_\_\_

|   |                             |
|---|-----------------------------|
| Test Name:  | Result & Range:             |
| CMV Viral load:<br>Quantitative Real Time PCR in plasma sample  | <i>7.8 x 10<sup>3</sup></i> |
| Real Time PCR kit Name: <i>Tru PCR kit</i><br>Nucleic Acid Extraction: <i>Nuclisense EasyMag extraction / Qiagen kit extraction</i> |                             |

**Interpretation:**

| Result in copy number                           | Interpretation   |
|---|--|
| Target not detected                             | CMV DNA not detected   |
| < 50 IU/ml                                      | CMV DNA detected less than 50 IU/ml. These results should be interpreted with caution.   |
| Between 50 IU/ml and 1.12x10 <sup>6</sup> IU/ml | Quantified results greater than or equal to 50 CMV IU/ml and less than or equal to 1.12x 10 <sup>6</sup> IU/ml are within the linear range of the assay. |
| >1.12x 10 <sup>6</sup> IU/ml                    | CMV DNA detected above the linear range of the assay.  |

Copies / ml = IU/ml × 0.78 (conversion factor)

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# Practical Diagnostic Algorithm ( ICU focus)

- Clinical suspicion : Hypoxemia + new infiltrates in immunocompromised host ( SOT/HCT/HIC/high dose immunosuppression)
- Send tests: BAL PCR for quantitative  $\pm$  culture  $\pm$  microscopy ( if feasible) + serum PCR
- Interpret results:
  - 1) BAL CMV > 500IU/ml  $\rightarrow$  strong evidence for disease ( probable)
  - 2) BAL low/borderline  $\rightarrow$  repeat test or use cell- normalized load if available
  - 3) Serum DNAemia alone  $\rightarrow$  not diagnostic , supportive only

# When to treat ( Practical rule)

Treat if ANY of the following:

- Proven CMV pneumonitis (tissue/IHC) — treat.
- Probable CMV pneumonitis: hypoxemia + compatible CT + BAL CMV high (>500 IU/mL) and no alternative cause — treat.
- Severe respiratory failure + high BAL viral load or progressive disease despite treatment of co-pathogens - treat empirically while awaiting results.
- Evidence shows CMV reactivation in ICU associates with worse outcomes (OR  $\approx$ 2.5 for mortality) — justify empiric therapy in selected critical patients.
- Do NOT treat low BAL DNAemia without clinical syndrome; instead monitor and repeat testing.

THANK YOU