Rigshospitalet

Faculty of Health and Medical Sciences

PERSIMUNE
CENTRE OF EXCELLENCE FOR PERSONALISED MEDICINE OF INFECTIOUS COMPLICATIONS IN IMMUNE DEFICIENCY

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

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MATCH
Management of Post-Transplant Infections in Collaborating Hospitals

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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]

1.Kotton, C.N. et al, Transplantation, 2013
2.Razonable RR et al, CID, 2013
3. Cummins NW et al, Transpl. Infect. Dis. 2009
4. Wiita AP, et al, Transpl. Infect. Dis. 2012

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

Certainty of diagnosis	Criteria for diagnosis of CMV pneumonia in lung transplant recipients
Proven	Relevant symptoms/signs of pneumonia + positive CMV histology in lung biopsy
Probable	Relevant symptoms/signs of pneumonia + positive CMV PCR in BALF + infiltrative changes on CT-scan (if available)

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

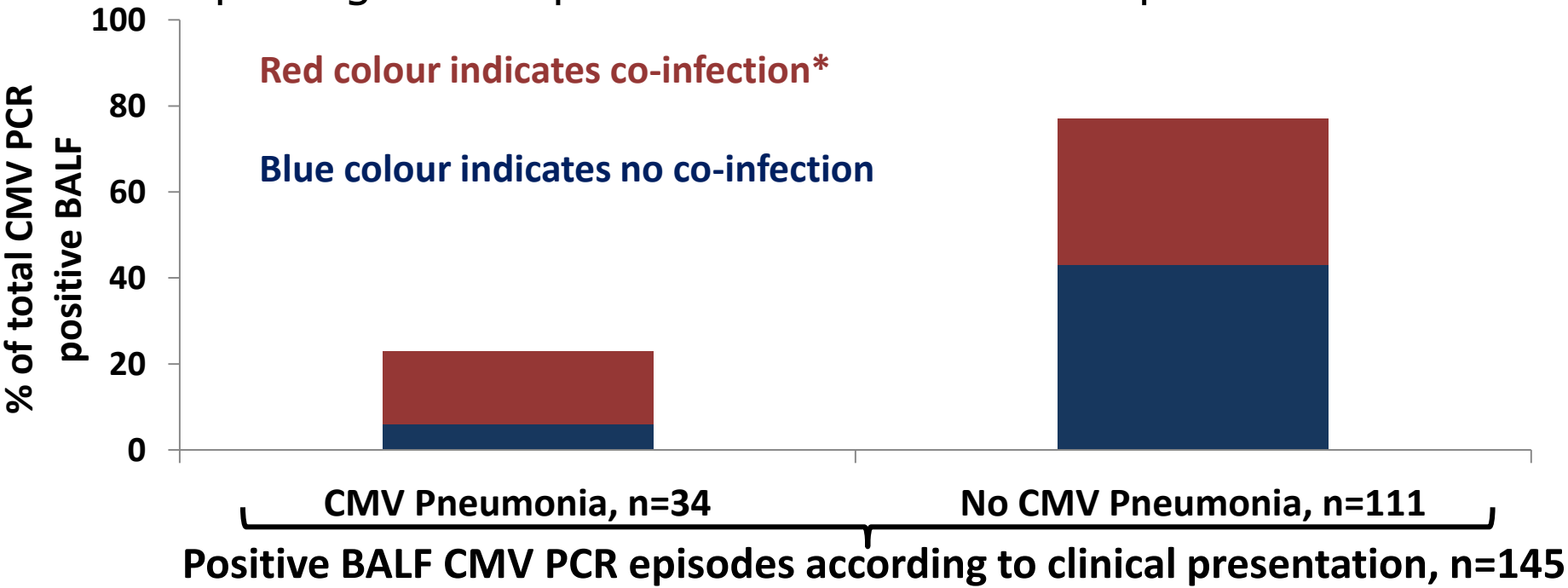
Statistical analyses

- 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

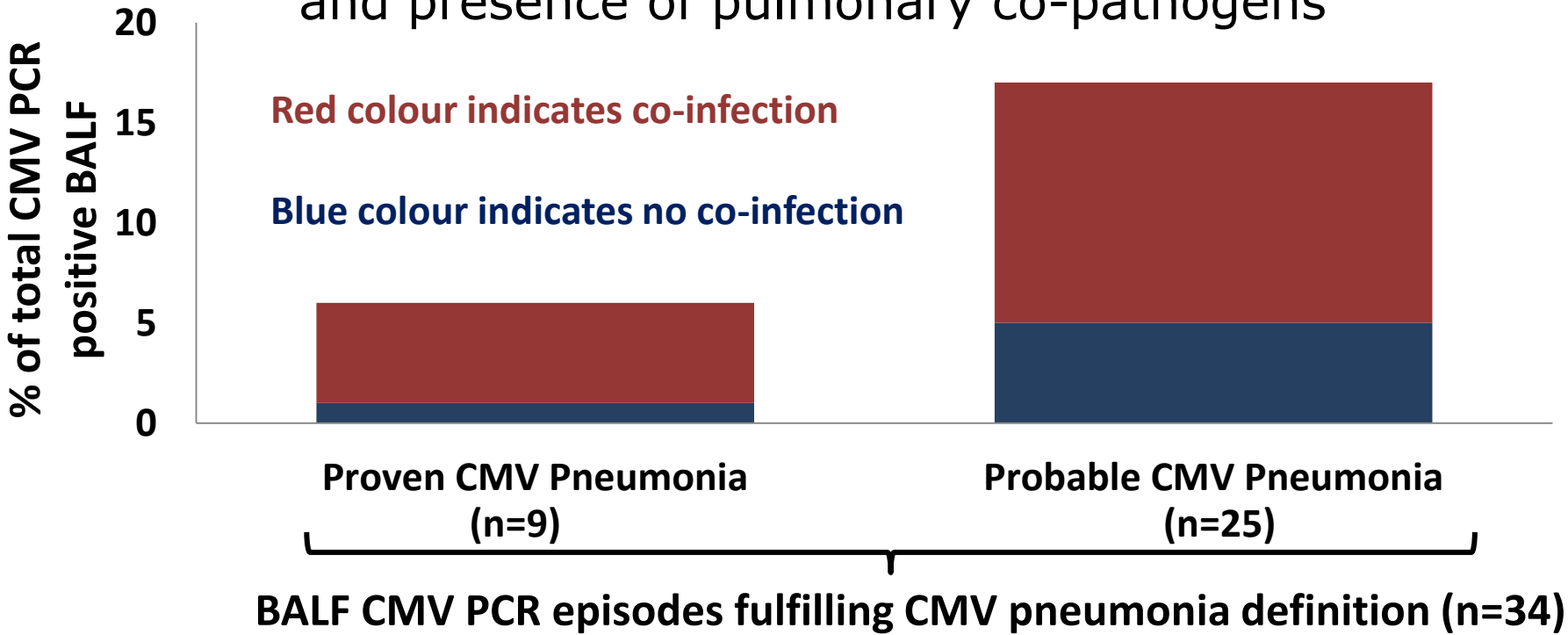
- A total of 141 recipients were transplanted during the study period
  - a total of 981 bronchoscopies were performed in this population in 1st year post-transplant
- 66 (47%) recipients had ≥ 1 CMV PCR positive BALF episode
  - Age, gender and distribution of CMV IgG sero-constellations were evenly 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

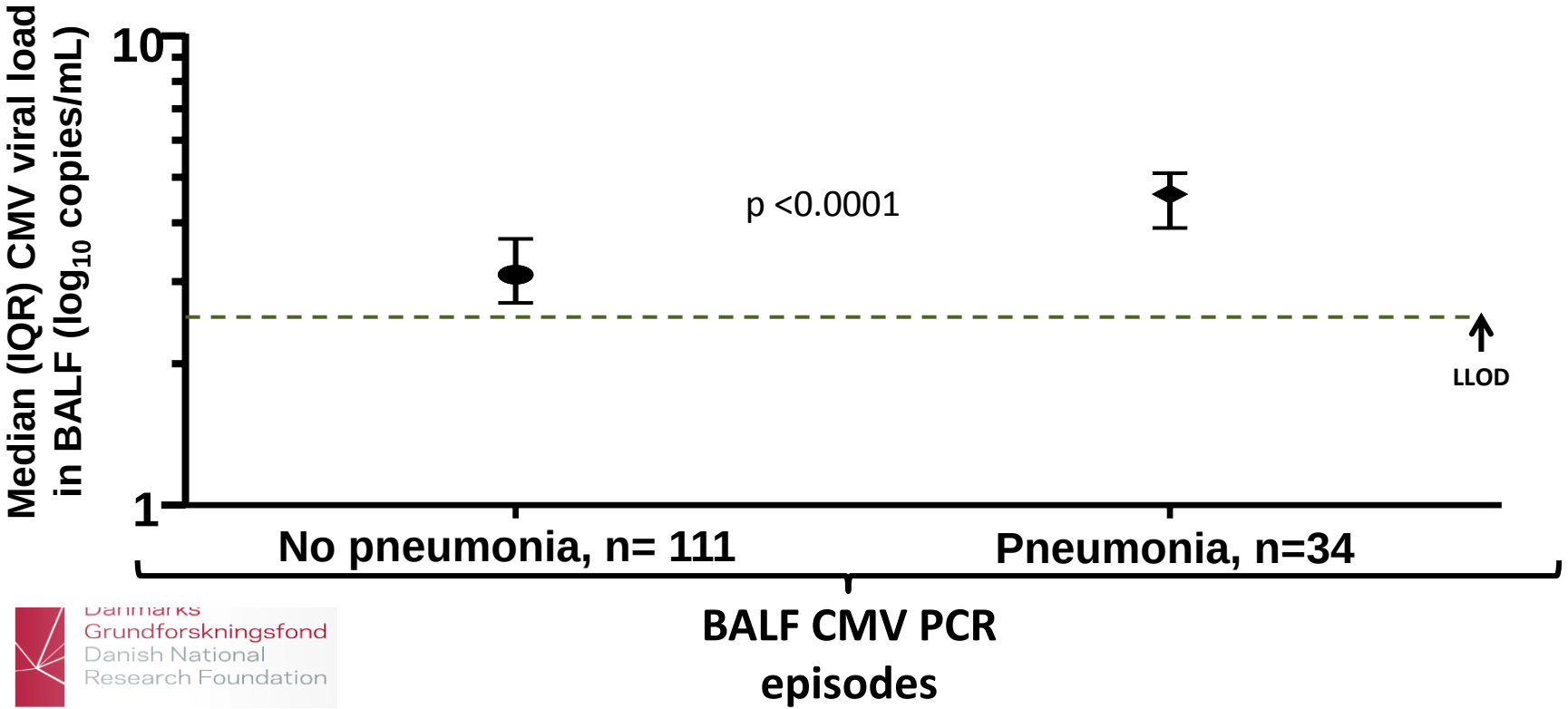


* Information on positive microbiology +/- 7 days within BALF was collected from medical records, and type of co-pathogen/s documented (fungal/bacterial/viral)

CMV pneumonia episodes by degree of certainty of diagnosis and presence of pulmonary co-pathogens



Comparison of BALF CMV viral load in episodes with and without CMV pneumonia



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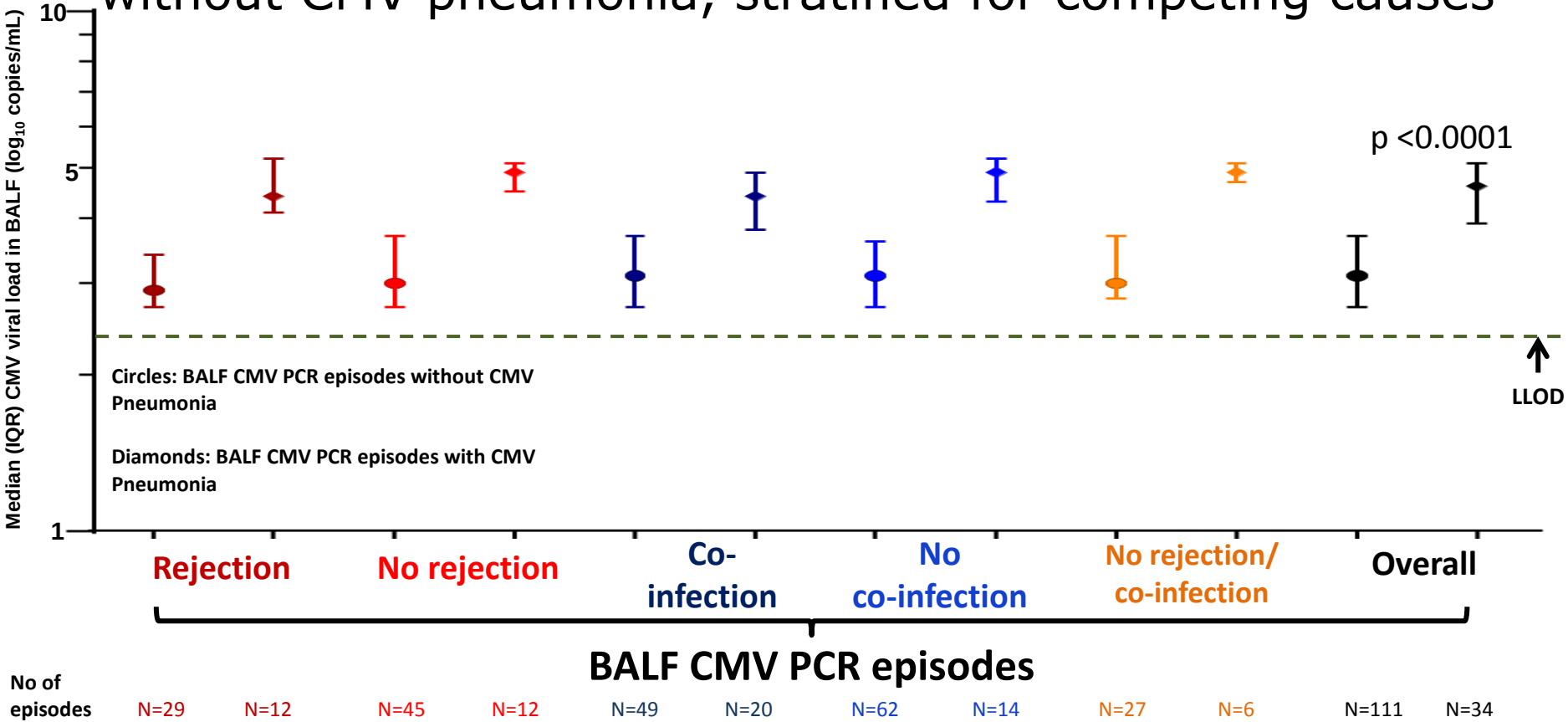
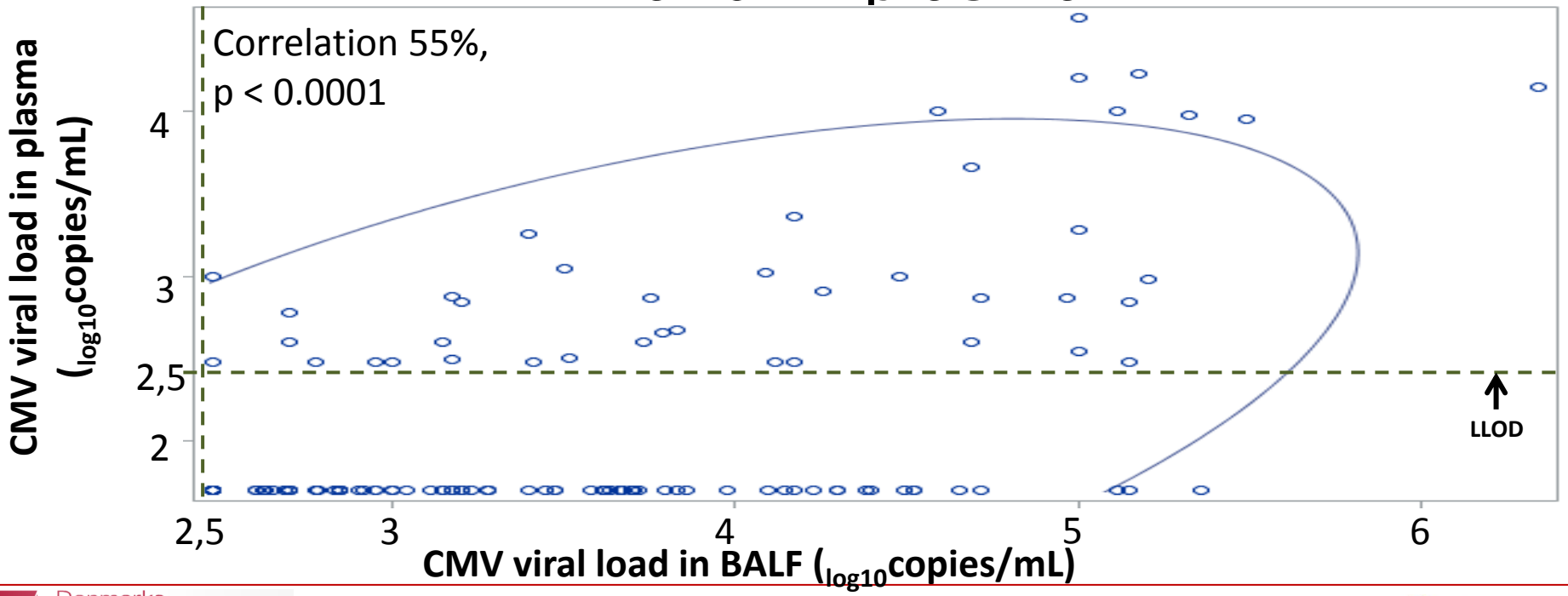
Comparison of BALF CMV viral load in episodes with and without CMV pneumonia, stratified for competing causes



Correlation of CMV viral load measured in BALF and in plasma



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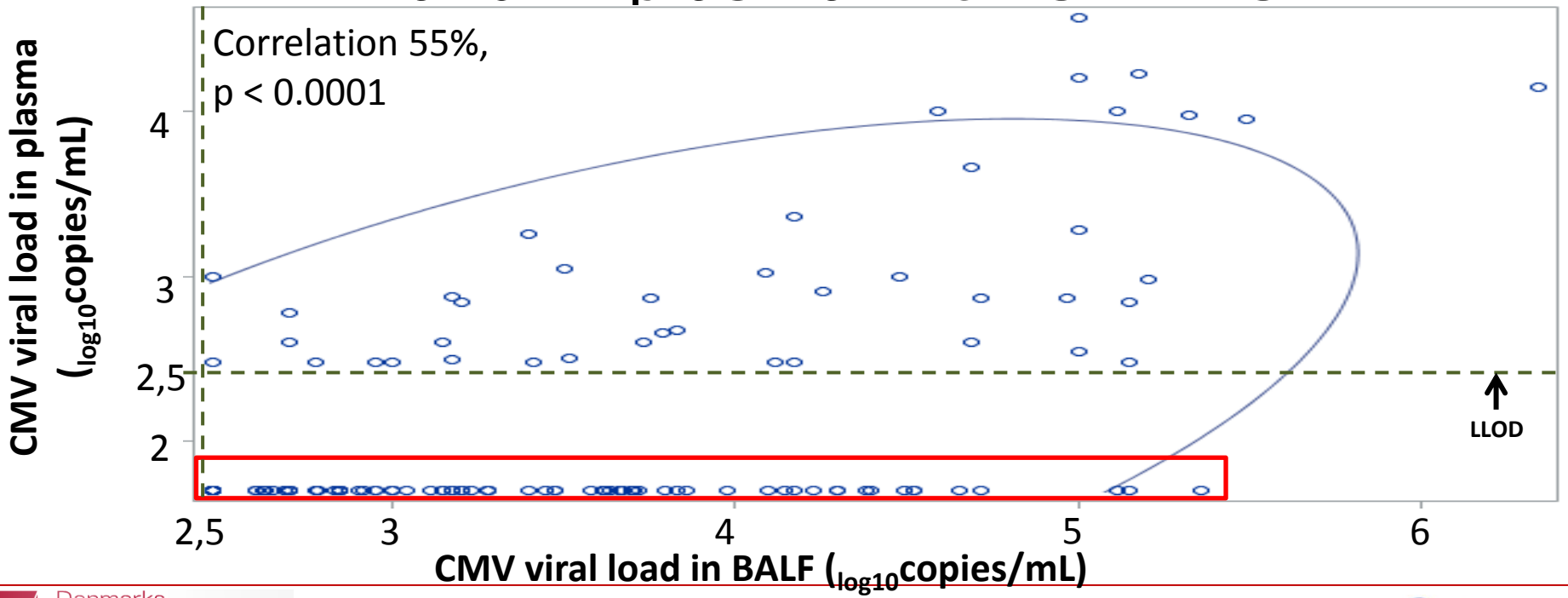
- Dashed green lines indicate lower limit of detection (LLOD) of CMV PCR kit at 300 copies/mL (=270 IU/mL)

Correlation of CMV viral load measured in BALF and in plasma with CMV PCR



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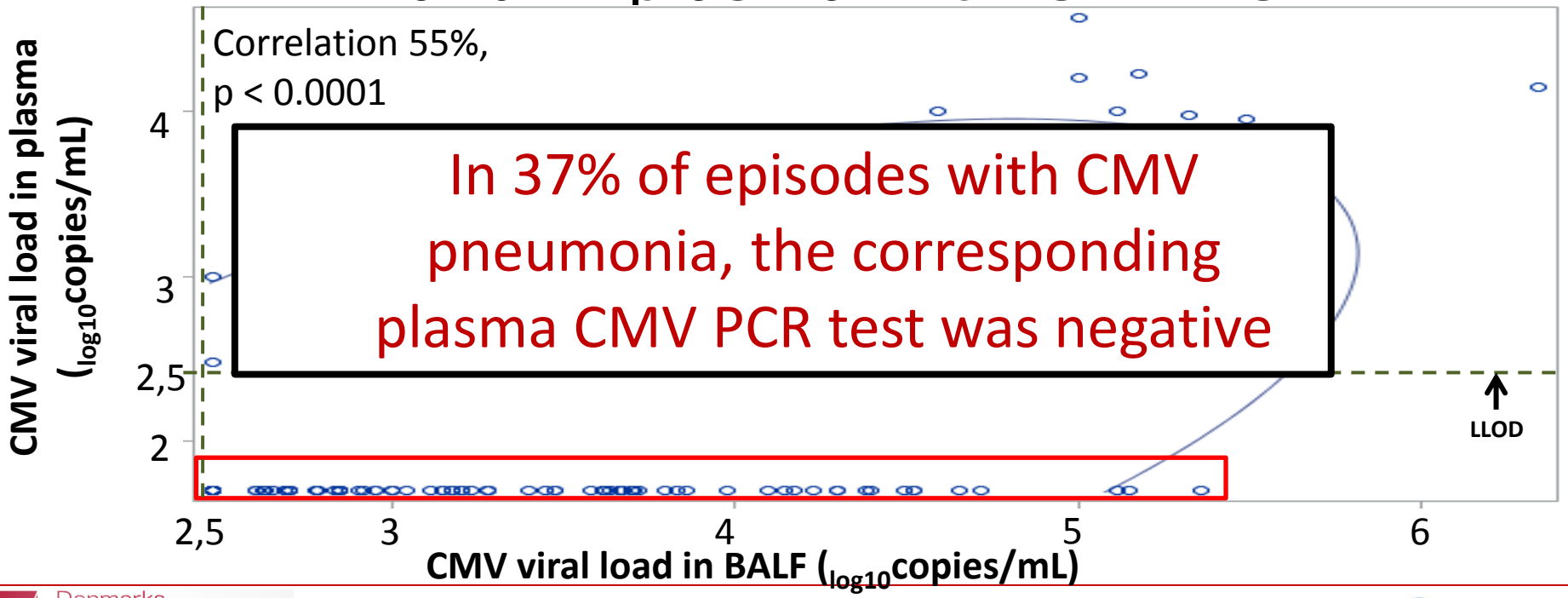
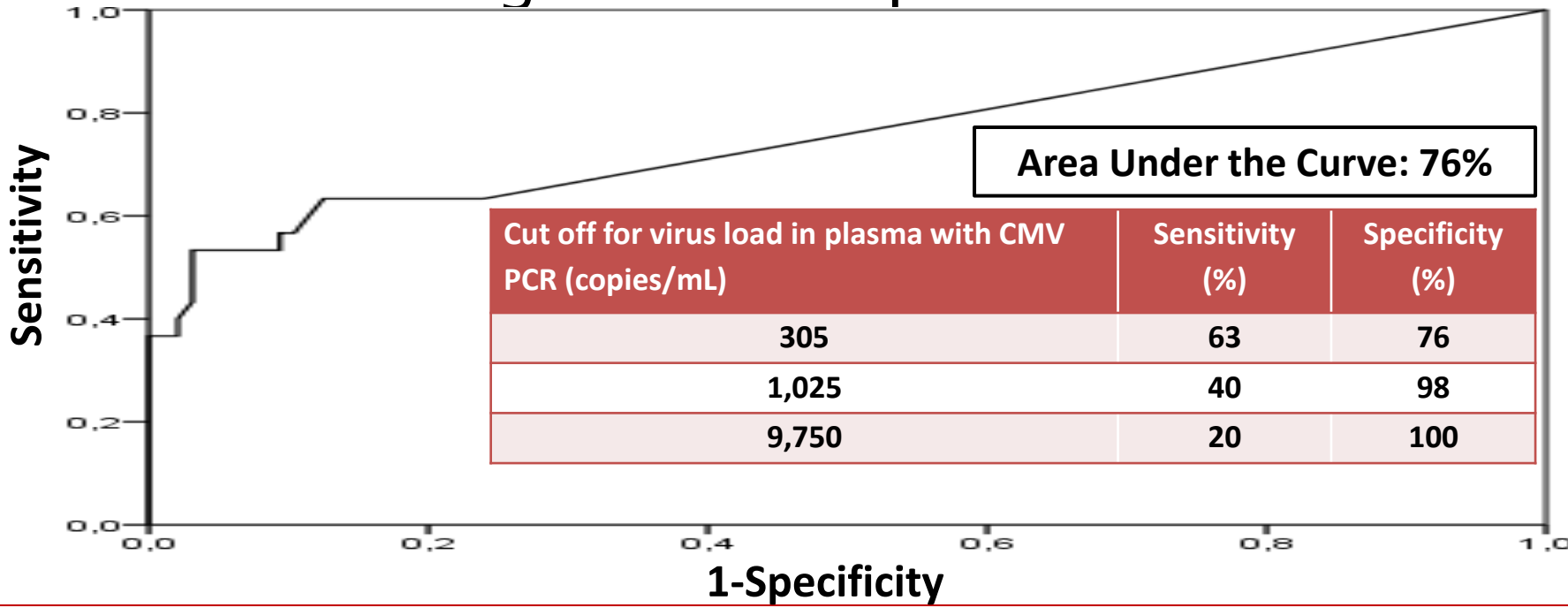
Correlation of CMV viral load measured in BALF and in plasma with CMV PCR



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

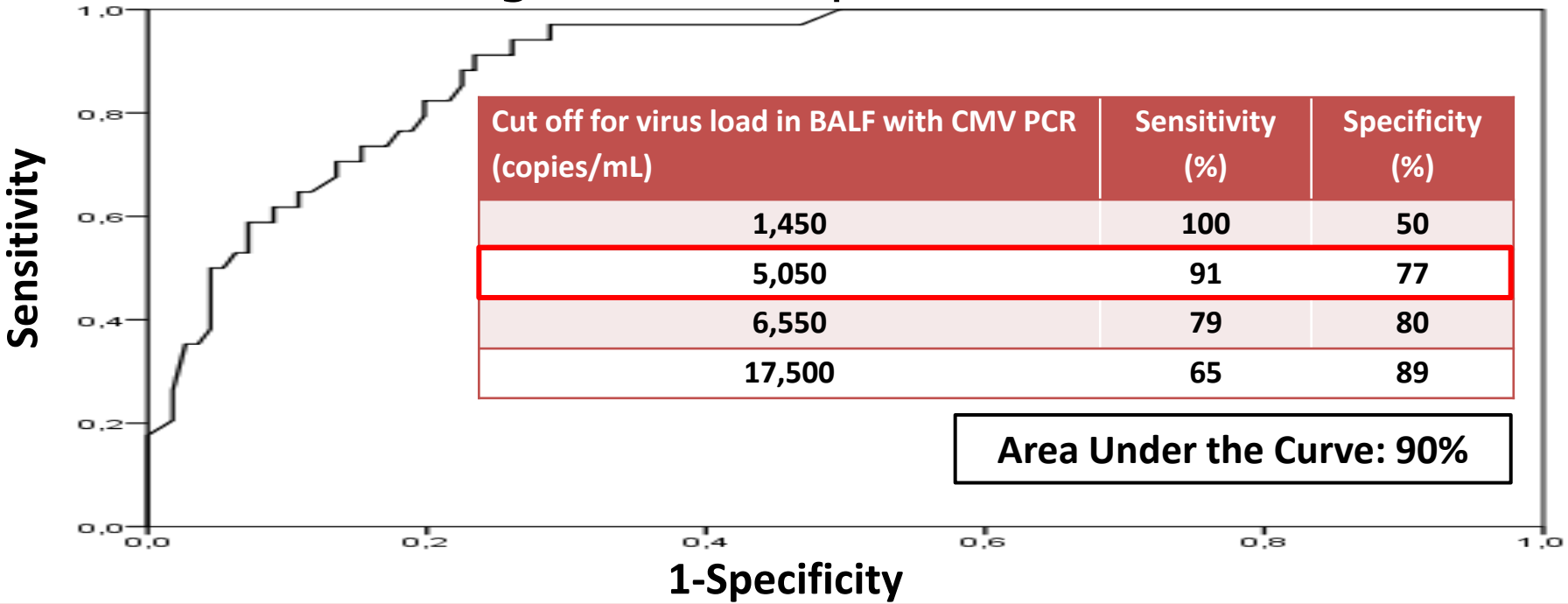
Diagnostic accuracy of CMV PCR in plasma to diagnose CMV pneumonia



Area Under the Curve: 76%

Cut off for virus load in plasma with CMV PCR (copies/mL)	Sensitivity (%)	Specificity (%)
305	63	76
1,025	40	98
9,750	20	100

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**

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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

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*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

Characteristics at BALF	CMV PNEUMONIA?												TOTAL n=145
	NO						YES						
	Overall, n=111	Rejection, n=29	No Rejection, n=45	Co-infection, n=49	No co-infection, n=62	No rejection or co-infection, n=27	Overall, n=34	Rejection, n=12	No Rejection, n=12	Co-infection, n=20	No co-infection, n=14	No rejection or co-infection, n=6	
% of BALF with biopsy*	67% 74/111	100% 29/29	100% 45/45	55% 27/49	76% 47/62	100% 27/27	70.5% 24/34	100% 12/12	100% 12/12	50% 10/20	100% 14/14	100% 6/6	67.5% 98/145
% with CMV detected in biopsy	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	37.5% 9/24	33% 4/12	42% 5/12	10% 2/20	50% 7/14	50% 3/6	9% 9/98
% with acute rejection in biopsy	39% 29/74	N.A.	N.A.	33% 9/27	42.5% 20/47	N.A.	50% 12/24	N.A.	N.A.	20% 4/20	50% 7/14	N.A.	42% 41/98
% with interstitial changes in biopsy*	44.5% 33/74	52% 15/29	40% 18/45	48% 13/27	42.5% 20/47	44% 12/27	50% 12/24	50% 6/12	50% 6/12	35 7/20	36% 5/14	33% 2/6	46% 45/98
% of BALF where CMV is the only detected pathogen**	56% 62/111	69% 20/29	60% 27/45	N.A.	100% 62/62	100% 27/27	41% 14/34	67% 8/12	50% 6/12	N.A.	100% 14/14	100% 6/6	76/145
Co-infections***													
% with fungal co-infection	34% 38/111	27.5% 8/29	31% 14/45	77.5% 38/49	N.A.	N.A.	32% 11/34	25% 3/12	17% 2/12	55% 11/20	N.A.	N.A.	34% 49/145
% with bacterial co-infection	21% 23/111	10% 3/29	18% 8/45	47% 23/49	N.A.	N.A.	41% 14/34	17% 2/12	42% 5/12	70% 14/20	N.A.	N.A.	25.5% 37/145
% with viral co infection	9% 10/111	0% 0/29	0% 0/45	10% 10/49	N.A.	N.A.	9% 3/34	0% 0/12	8% 1/12	15% 3/20	N.A.	N.A.	9% 13/145
Median time from tx to BALF (IQR)	169 (89-216)	175 (90-214)	187 (89-214)	145 (87-201)	178.5 (106-218)	187 (89-218)	149 (101-189)	127.5 (61-239)	164 (118.5-215.5)	145.5 (87-180.5)	149 (112-267)	170 (147-242)	155 (90-214)
Median VL in BALF CMV PCR (IQR)	1,400 (500-4,700)	830 (500-2,500)	1,000 (500-4,700)	1,400 (500-4,700)	1,400 (500-4,200)	1,000 (700-5,400)	36,000 (8,700-130,000)	25,500 (13,100-170,000)	74,500 (34,500-135,000)	24,000 (5,850-76,000)	74,500 (18,000-150,000)	74,500 (49,000-140,000)	2,500 (700-15,000)
Proportion of BALF with CMV PCR in plasma****	86% 96/111	90% 26/29	82% 37/45	88% 43/49	85% 53/62	81% 22/27	88% 30/34	92% 11/12	92% 11/12	80% 16/20	100% 14/14	100% 6/6	87% 126/145
% with positive CMV PCR	24% 23/96	11.5% 3/26	13.5% 5/37	25.5% 11/43	23% 12/53	14% 3/22	63% 19/30	54.5% 6/11	82% 9/11	56% 9/16	71% 10/14	83% 5/6	33% 42/126
Median VL in positive plasma CMV PCR (IQR)	400 (300-730)	1,000 (400-1,800)	450 (350-600)	600 (300-730)	330 (300-575)	350 (310-450)	2,300 (800-10,000)	2,100 (1,050-9,500)	10,000 (4,600-16,000)	1,000 (750-9,000)	7,050 (1,050-14,000)	10,000 (4,600-17,000)	730 (350-1,900)

*B-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