

Supplementary material

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Figure S1

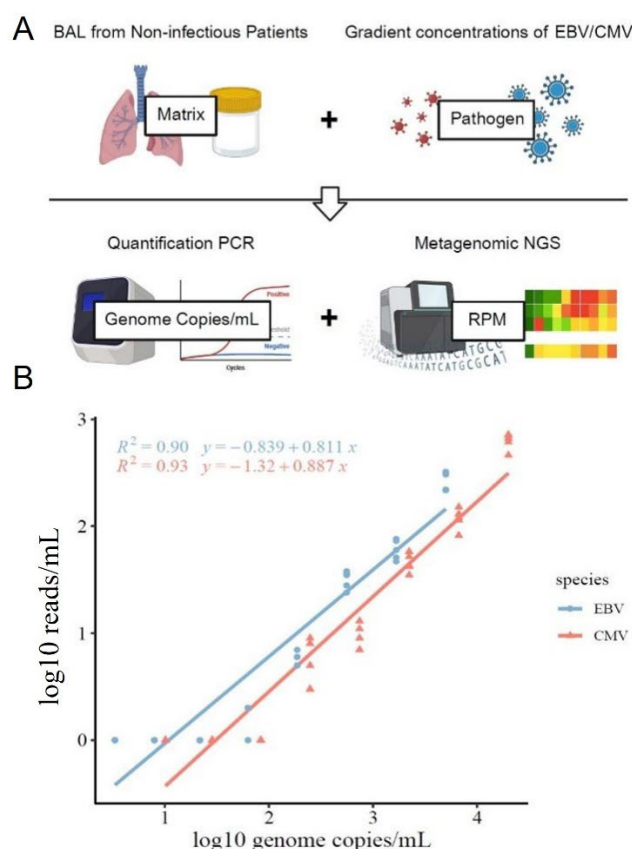


Figure S1. Performance Evaluation of Pathogen Detection Using Spiked BAL Samples

It illustrates the schematic workflow (Figure S1A) of the experimental design for assessing pathogen detection in bronchoalveolar lavage (BAL) samples from non-infectious patients. The BAL samples served as a matrix and were spiked with gradient concentrations of Epstein–Barr virus (EBV) and cytomegalovirus (CMV) DNA to simulate varying pathogen loads. These spiked samples underwent quantitative PCR (qPCR) for genome copy number quantification and metagenomic next-generation sequencing (mNGS) to measure relative pathogen abundance in reads per million (RPM). This dual-platform approach enabled a comparative analysis of pathogen quantification across different techniques^{1,2}.

A strong linear correlation was observed between the $\log_{10}$ -transformed genome copies per milliliter ($\log_{10}$ genome copies/mL) determined by qPCR and the $\log_{10}$ -transformed RPM from mNGS for both EBV and CMV (Figure S1B). For EBV, the linear regression equation was $y = -0.839 + 0.811 x$, with a coefficient of determination (R^2) of 0.90, indicating a strong correlation between the two quantification methods. For CMV, the linear regression equation was $y = -1.32 + 0.887 x$, with $R^2 = 0.93$, demonstrating an even stronger correlation. These findings underscore the reliability of mNGS in reflecting pathogen load, as quantified by qPCR, across a gradient of pathogen concentrations.

1. Diao Z, Lai H, Han D, et al. Validation of a Metagenomic Next-Generation Sequencing Assay for Lower Respiratory Pathogen Detection. *Microbiol Spectr.* 2023 Feb 14;11(1):e0381222.
2. Miller S, Naccache SN, Samayoa E, et al. Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid. *Genome Res.* 2019 May;29(5):831-842.

Figure S2

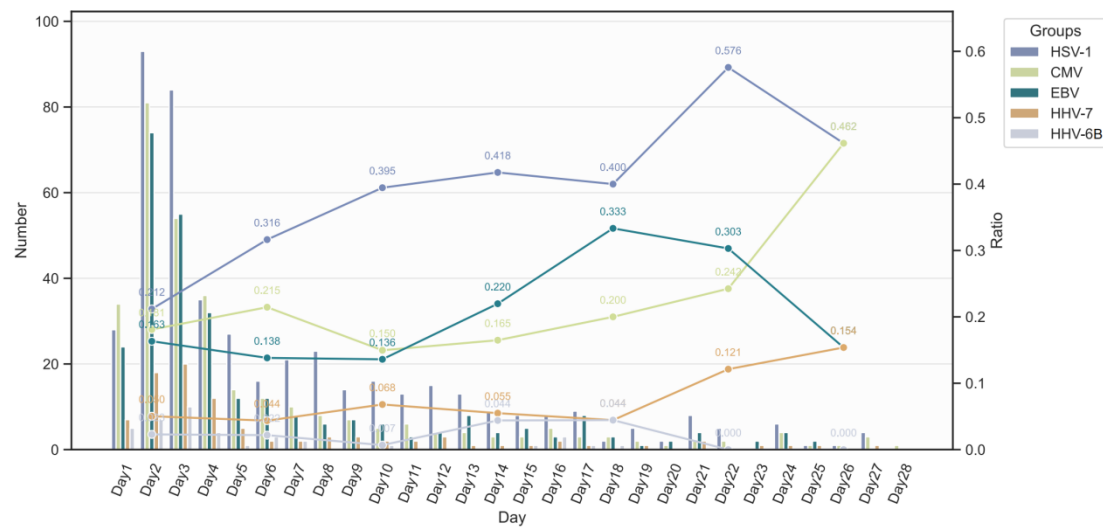


Figure S2. Number and proportion of patients initially herpesvirus positivity at different time points after ICU admission.

The x-axis represents the time points after ICU admission when clinical metagenomics testing was initiated. The x-axis represents the time points after ICU admission when clinical metagenomics testing was initiated. The left y-axis shows the daily number of patients testing positive for herpesvirus by mNGS, while the right y-axis indicates the proportion of patients testing positive for herpesvirus among those tested by mNGS over a 4-day period.

Figure S3

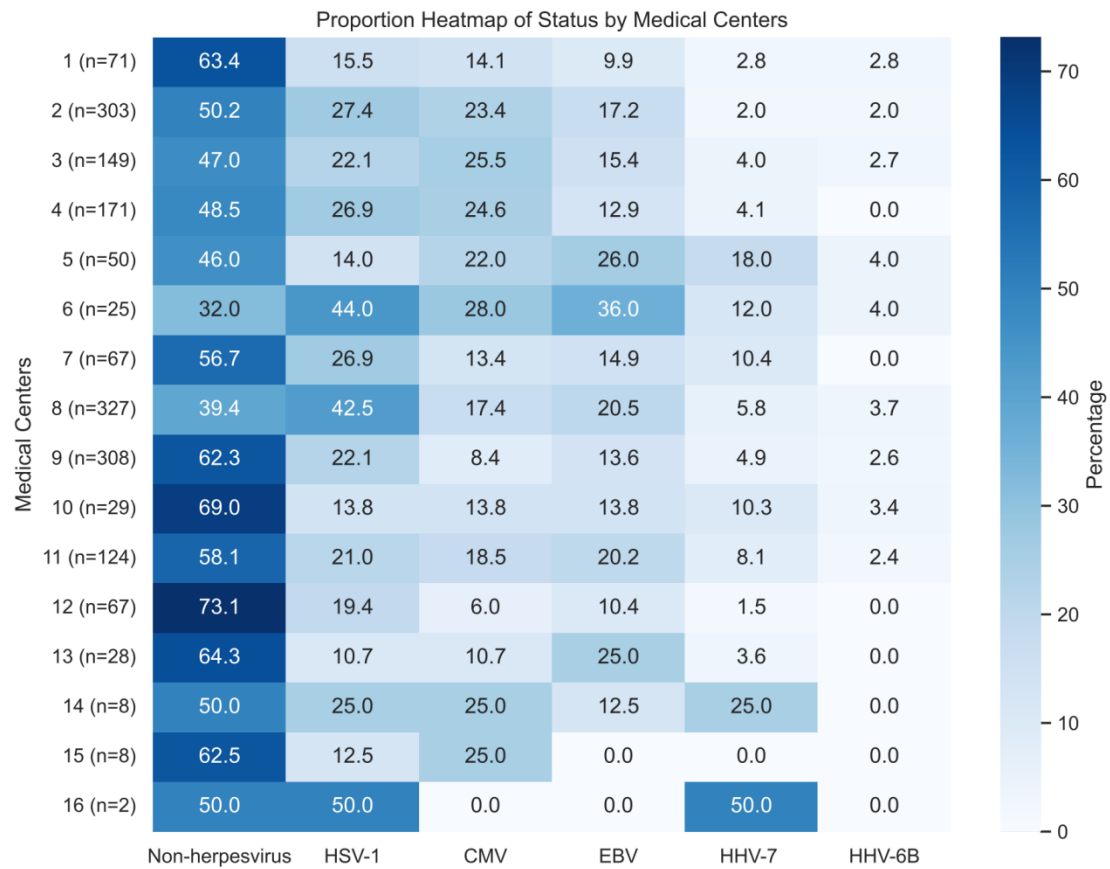


Figure legend:

Figure S3. Heatmap of herpesvirus distribution across different participant medical centers.

Two of the medical centers belong to the same medical group, and their data has been merged (Unit 13).

The percentage in each cell represents the proportion of patients at each medical center who tested positive for different herpesviruses. Since some patients may be detected with multiple herpesviruses simultaneously, the sum of the proportions for each center may exceed 100%.

Figure S4

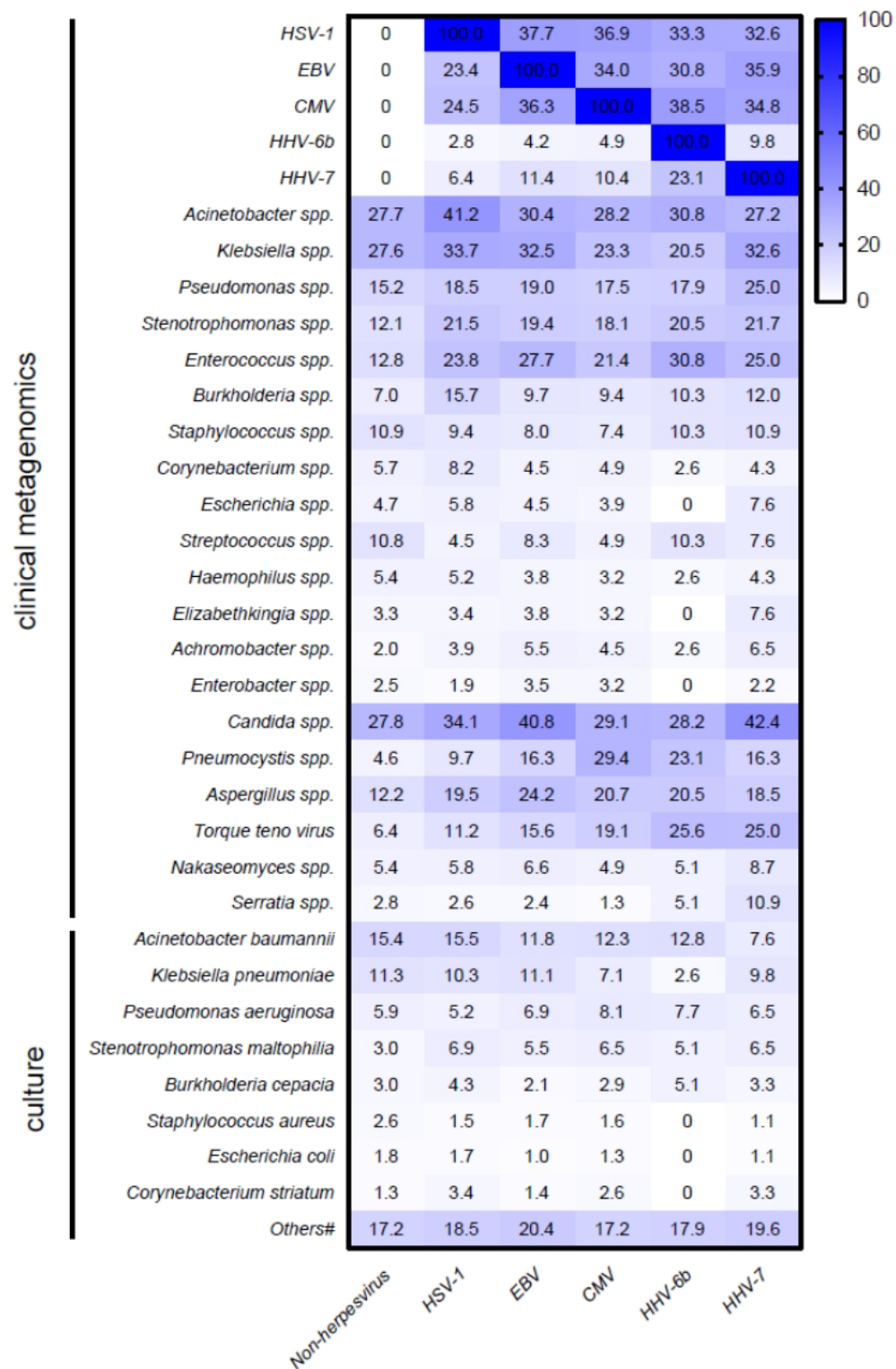


Figure legend:

Figure S4. Suspected pathogenic microorganisms detected by culture or clinical metagenomics in different groups.

Figure S5

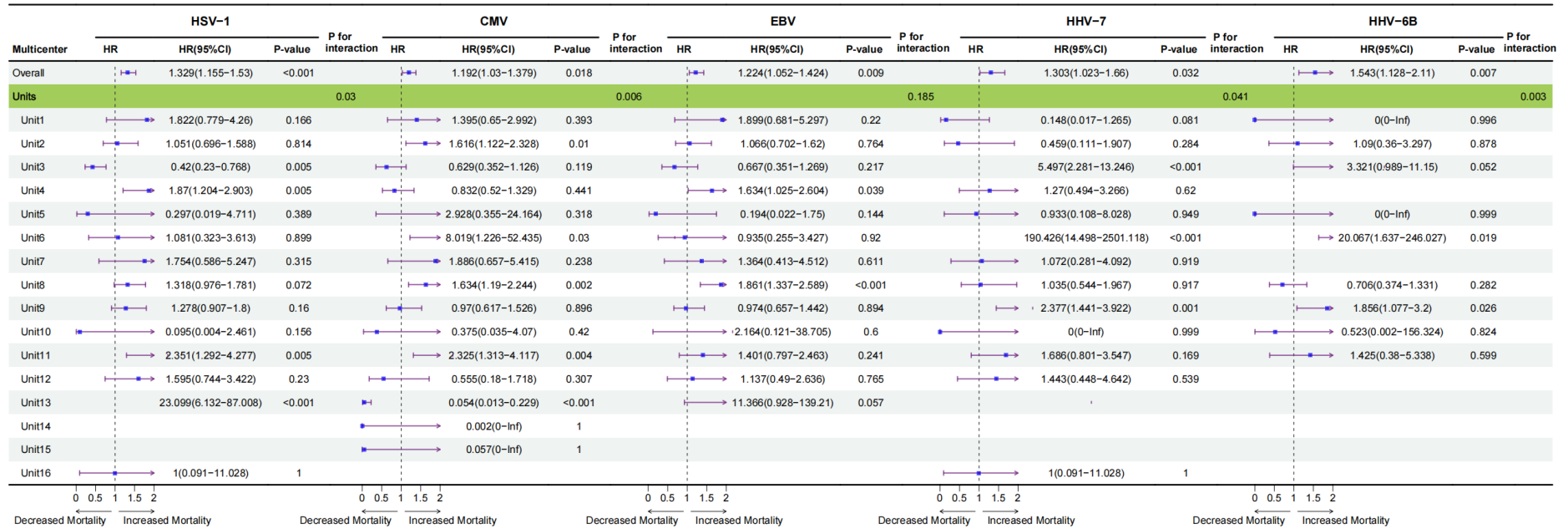


Figure legend:

Figure S5. Subgroup analysis of herpesviruses in different medical centers.

Table S1. Clinical characteristics of different herpesviruses in patients with community-acquired pneumonia

Variables	Non-herpesvirus (n=561)	HSV-1 (n=265)	CMV (n=191)	EBV (n=190)	HHV-7 (n=50)	HHV-6B (n=21)	<i>P</i> value
Age, years, median (IQR)	68 (56, 76)	67 (59, 76)	66 (56, 76)	69 (62, 77)	61.5 (47.5, 71.8) ^d	60 (48, 73)	0.014
Male gender, n (%)	377 (67.2)	193 (72.8)	136 (71.2)	133 (70.0)	41 (82.0)	15 (71.4)	0.245
Comorbidities, n (%)							
Diabetes mellitus	165 (29.4)	64 (24.2)	51 (26.7)	41 (21.6)	9 (18.0)	5 (23.8)	0.187
Myocardial infarction	41 (7.3)	14 (5.3)	9 (4.7)	8 (4.2)	2 (4.0)	1 (4.8)	0.544
Chronic pulmonary disease	117 (20.9)	66 (24.9)	47 (24.6)	47 (24.7)	8 (16.0)	3 (14.3)	0.441
Liver disease	21 (3.7)	29 (10.9) ^a	19 (9.9) ^a	14 (7.4)	2 (4.0)	0 (0.0)	<0.001
Chronic kidney disease	71 (12.7)	38 (14.3)	45 (23.6) ^a	35 (18.4)	10 (20.0)	1 (4.8)	0.005
Solid tumor	71 (12.7)	32 (12.1)	31 (16.2)	29 (15.3)	7 (14.0)	2 (9.5)	0.726
Hematologic malignancy	20 (3.6)	11 (4.2)	11 (5.8)	8 (4.2)	1 (2.0)	2 (9.5)	0.573
Connective tissue disease	22 (3.9)	10 (3.8)	20 (10.5) ^a	19 (10.0) ^a	3 (6.0)	3 (14.3)	<0.001
Transplantation	23 (4.1)	13 (4.9)	17 (8.9)	20 (10.5) ^a	2 (4.0)	2 (9.5)	0.012
Cerebrovascular disease	103 (18.4)	38 (14.3)	28 (14.7)	29 (15.3)	10 (20.0)	7 (33.3)	0.184
Immunosuppression, n (%)	114 (20.3)	80 (30.2) ^a	88 (46.1) ^{ab}	80 (42.1) ^a	14 (28.0)	10 (47.6)	<0.001
Respiratory support, n (%)							0.002
IMV	491 (87.5)	216 (81.5)	163 (85.3)	156 (82.1)	33 (66.0) ^a	14 (66.7)	
NIV	18 (3.2)	11 (4.2)	8 (4.2)	7 (3.7)	4 (8.0) ^a	3 (14.3)	

Others [#]	52 (9.3)	38 (14.3)	20 (10.5)	27 (14.2)	13 (26.0) ^a	4 (19.0)	
Duration of IMV, median (IQR)	8 (3, 14)	7 (2, 15)	8 (3, 14)	7 (2, 12)	5 (0, 8) ^{ac}	8 (0, 19)	0.022
Ventilator-free days at day 28, median (IQR)	1 (0, 18)	1 (0, 12)	1 (0, 9.5)	1 (0, 13.5)	0 (0, 9.2)	0 (0, 1)	0.023
Laboratory indicators, median (IQR)							
White blood cell ($\times 10^9/L$)	11.1 (6.5, 15.6)	11.7 (7.3, 17.0)	10.3 (6.5, 14.5)	11.2 (7.3, 15.7)	10.3 (7.1, 14.3)	10.0 (7.5, 15.8)	0.479
Lymphocyte ($\times 10^9/L$)	0.6 (0.3, 0.9)	0.5 (0.3, 0.9)	0.4 (0.2, 0.7) ^a	0.5 (0.3, 0.9)	0.5 (0.3, 0.9)	0.4 (0.2, 0.8)	<0.001
Neutrophil ($\times 10^9/L$)	9.7 (5.5, 13.9)	10.3 (6.2, 15.0)	9.0 (5.8, 13.5)	10.0 (6.1, 14.3)	9.1 (5.9, 12.4)	9.5 (6.6, 14.3)	0.703
C-reactive protein (mg/L)	105.8 (40.7, 184.7)	84.0 (36.5, 145.3)	91.2 (42.2, 147.3)	95.4 (40.9, 146.7)	102.3 (47.0, 141.8)	90.9 (30.6, 163.1)	0.158
Procalcitonin (ng/ml)	1.2 (0.2, 9.8)	0.7 (0.2, 2.5)	0.8 (0.2, 2.9)	0.8 (0.2, 2.8)	0.6 (0.3, 2.1)	0.4 (0.1, 1.5)	0.023
SOFA at the time of ICU admission, median (IQR)	7 (4, 10)	7 (4, 9)	7 (4, 9)	7 (4, 9)	6 (3.2, 9)	6 (4, 9)	0.306
SOFA at the time of mNGS testing, median (IQR)	8 (5, 10)	7 (5, 10)	8 (5, 11)	7 (5, 10)	7 (4, 9)	5 (4, 8)	0.212
Organ dysfunction at the time of mNGS testing, n (%)							
Respiratory	493 (87.9)	220 (83.0)	166 (86.9)	168 (88.4)	40 (80.0)	18 (85.7)	0.297
Coagulation	169 (30.1)	104 (39.2)	75 (39.3)	68 (35.8)	17 (34.0)	7 (33.3)	0.091
Hepatic	68 (12.1)	44 (16.6)	28 (14.7)	25 (13.2)	8 (16.0)	1 (4.8)	0.425
Cardiovascular	310 (55.3)	126 (47.5)	107 (56.0)	94 (49.5)	16 (32.0) ^a	6 (28.6)	0.002
Neurologic	204 (36.4)	95 (35.8)	62 (32.5)	60 (31.6)	21 (42.0)	9 (42.9)	0.601
Kidney	134 (23.9)	49 (18.5)	55 (28.8)	48 (25.3)	13 (26.0)	4 (19.0)	0.194

Results of clinical metagenomics [†]							
Reads, median (IQR)	/	182.0 (14.0, 5075.0)	31.0 (8.0, 217.0) ^b	21.0 (5.0, 136.8) ^b	8.0 (4.0, 32.0) ^{bc}	21.0 (7.0, 80.0) ^b	<0.001
≥100 (Reactivation), n (%)	/	145 (54.7)	59 (31.1) ^b	53 (27.9) ^b	5 (10.0) ^{bc}	4 (19.1) ^b	<0.001
<100 (Detection), n (%)	/	120 (45.3)	131 (67.0) ^b	137 (72.1) ^b	45 (90.0) ^{bc}	17 (81.0) ^b	
Missing data, n (%)	/	/	1 (0.5)	/	/	/	
Anti-herpesvirus, n (%)							
Ganciclovir	10 (1.8)	19 (7.2) ^a	34 (17.8) ^{ab}	16 (8.4) ^a	1 (2.0)	2 (9.5)	<0.001
Acyclovir	4 (0.7)	23 (8.7) ^a	6 (3.1)	6 (3.2)	1 (2.0)	0 (0.0)	<0.001
Others [§]	1 (0.2)	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.401
Corticosteroid	335 (59.7)	145 (54.7)	138 (72.3) ^{ab}	116 (61.1)	30 (60.0)	12 (57.1)	0.010
Hospital stays, median (IQR)	18 (10, 29)	19 (10, 34)	17 (9, 29)	16 (9, 30)	16.5 (8.5, 28.8)	20 (10, 36)	0.447
ICU stays, median (IQR)	12 (7, 21)	14 (7, 23)	11 (7, 20)	10 (7, 19)	9 (6, 16.8)	11 (8, 21)	0.106
Time from ICU admission to mNGS testing, median (IQR)	2 (2, 4)	3 (2, 7) ^a	3 (2, 5)	3 (2, 5)	3 (2, 4)	3 (2, 6)	<0.001
28-day mortality, n (%)	242 (43.1)	120 (45.3)	98 (51.3)	93 (48.9)	21 (42.0)	10 (47.6)	0.411

Notes:

Pairwise tests were performed for significant variables, with Bonferroni correction to control false positives. In the table, the symbols ^a, ^b, ^c, ^d, and ^e indicate statistical significance between groups, where these symbols represent an adjusted *P* value < 0.05.

^a Compared with non-herpesvirus;

^b Compared with HSV-1;

^c Compared with CMV;

^d Compared with EBV;

^e Compared with HHV-7.

[#] Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

[†] Statistical analysis of clinical metagenomics results was performed among five groups of herpesvirus patients, with one case excluded due to missing reads data.

[§] Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviations: HSV-1, Herpes simplex virus-1; EBV, Epstein-Barr virus; CMV, Cytomegalovirus; HHV-6B, Human herpesvirus-6B; HHV-7, Human herpesvirus-7; IQR, interquartile range; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Table S2. Clinical characteristics of different herpesviruses in patients with hospital-acquired pneumonia

Variables	Non-herpesvirus (n=212)	HSV-1 (n=104)	CMV (n=85)	EBV (n=67)	HHV-7 (n=30)	HHV-6B (n=12)	<i>P</i> value
Age, years, median (IQR)	65 (55, 73.2)	67 (56, 76.2)	68 (57, 75)	67 (56.5, 75)	65.5 (51.5, 76.5)	54 (48, 63)	0.559
Male gender, n (%)	153 (72.2)	67 (64.4)	61 (71.8)	50 (74.6)	23 (76.7)	5 (41.7)	0.152
Comorbidities, n (%)							
Diabetes mellitus	43 (20.3)	18 (17.3)	16 (18.8)	18 (26.9)	6 (20.0)	2 (16.7)	0.764
Myocardial infarction	17 (8.0)	5 (4.8)	5 (5.9)	2 (3.0)	1 (3.3)	1 (8.3)	0.658
Chronic pulmonary disease	36 (17.0)	16 (15.4)	17 (20.0)	6 (9.0)	3 (10.0)	2 (16.7)	0.479
Liver disease	19 (9.0)	10 (9.6)	12 (14.1)	7 (10.4)	1 (3.3)	0 (0.0)	0.443
Chronic kidney disease	22 (10.4)	14 (13.5)	15 (17.6)	15 (22.4)	3 (10.0)	0 (0.0)	0.083
Solid tumor	43 (20.3)	22 (21.2)	17 (20.0)	15 (22.4)	6 (20.0)	0 (0.0)	0.654
Hematologic malignancy	20 (9.4)	6 (5.8)	15 (17.6)	5 (7.5)	3 (10.0)	4 (33.3)	0.011
Connective tissue disease	9 (4.2)	3 (2.9)	3 (3.5)	5 (7.5)	1 (3.3)	2 (16.7)	0.271
Transplantation	8 (3.8)	3 (2.9)	11 (12.9)	4 (6.0)	1 (3.3)	0 (0.0)	0.054
Cerebrovascular disease	35 (16.5)	19 (18.3)	13 (15.3)	10 (14.9)	5 (16.7)	0 (0.0)	0.729
Immunosuppression, n (%)	57 (26.9)	25 (24.0)	35 (41.2)	20 (29.9)	12 (40.0)	7 (58.3)	0.019
Respiratory support, n (%)							0.023
IMV	181 (85.4)	85 (81.7)	71 (83.5)	43 (64.2) ^a	24 (80.0)	9 (75.0)	
NIV	8 (3.8)	1 (1.0)	3 (3.5)	5 (7.5) ^a	1 (3.3)	1 (8.3)	
Others [#]	23 (10.8)	18 (17.3)	11 (12.9)	19 (28.4) ^a	5 (16.7)	2 (16.7)	

Time from admission to HAP onset, median (IQR)	7 (3, 14)	11 (4.8, 17.2)	11 (3, 23)	12 (3, 19)	8 (4.2, 19.5)	13.5 (6.5, 19)	0.039
Duration of IMV, median (IQR)	8 (3, 17)	8.5 (2, 16.2)	8 (3, 14)	3 (0, 9.5) ^{abc}	8 (2.5, 14.8)	4 (0.8, 9)	0.003
Ventilator-free days at day 28, median (IQR)	2 (0, 17)	1 (0, 14.2)	1 (0, 17)	1 (0, 16.5)	2 (0, 19.5)	2 (0.8, 18)	0.850
Laboratory indicators, median (IQR)							
White blood cell ($\times 10^9/L$)	9.7 (6.3, 14.0)	10.6 (7.1, 15.2)	11.4 (6.9, 16.4)	9.9 (6.2, 13.8)	10.8 (8.7, 14.4)	9.7 (3.8, 12.8)	0.481
Lymphocyte ($\times 10^9/L$)	0.6 (0.3, 1.1)	0.7 (0.3, 1.2)	0.7 (0.3, 1.0)	0.7 (0.3, 1.1)	0.8 (0.4, 1.1)	0.7 (0.2, 1.3)	0.910
Neutrophil ($\times 10^9/L$)	8.5 (4.7, 12.5)	9.4 (5.2, 13.4)	9.8 (5.4, 14.9)	8.1 (4.8, 12.1)	9.6 (5.7, 13.1)	5.2 (1.9, 10.2)	0.277
C-reactive protein (mg/L)	73.8 (23.7, 125.4)	100.7 (24.0, 190.7)	79.8 (35.6, 153.5)	94.3 (30.7, 161.2)	62.8 (29.6, 185.5)	81.5 (26.4, 215.6)	0.719
Procalcitonin (ng/ml)	0.7 (0.2, 4.5)	1.4 (0.4, 7.6)	1.1 (0.3, 4.1)	1.9 (0.6, 9.8)	0.7 (0.2, 8.4)	1.1 (0.2, 6.5)	0.175
SOFA at the time of ICU admission, median (IQR)	6 (4, 9)	6 (4, 9)	7 (4, 8)	6 (3.5, 9)	5 (4, 7)	5 (3.8, 7)	0.723
SOFA at the time of mNGS testing, median (IQR)	7 (5, 10)	7 (5, 10)	7 (5, 10)	7 (4, 11)	6 (4, 8)	6.5 (3.8, 8.8)	0.264
Organ dysfunction at the time of mNGS testing, n (%)							
Respiratory	176 (83.0)	86 (82.7)	70 (82.4)	51 (76.1)	18 (60.0)	7 (58.3)	0.018
Coagulation	76 (35.8)	47 (45.2)	36 (42.4)	30 (44.8)	13 (43.3)	7 (58.3)	0.404
Hepatic	41 (19.3)	20 (19.2)	17 (20.0)	12 (17.9)	2 (6.7)	1 (8.3)	0.563
Cardiovascular	109 (51.4)	52 (50.0)	39 (45.9)	26 (38.8)	9 (30.0)	5 (41.7)	0.186
Neurologic	74 (34.9)	36 (34.6)	21 (24.7)	20 (29.9)	10 (33.3)	3 (25.0)	0.601
Kidney	41 (19.3)	26 (25.0)	20 (23.5)	22 (32.8)	3 (10.0)	3 (25.0)	0.133

Results of clinical metagenomics [†]							
Reads, median (IQR)	/	220.5 (21.8, 6536.0)	22.0 (5.0, 171.0) ^b	28.0 (8.0, 119.0) ^b	9.5 (4.0, 54.5) ^b	13.0 (4.5, 120.5)	<0.001
≥100 (Reactivation), n (%)	/	56 (53.9)	26 (30.6) ^b	18 (26.9) ^b	4 (13.3) ^b	3 (25.0)	<0.001
<100 (Detection), n (%)	/	48 (46.2)	59 (69.4) ^b	49 (73.1) ^b	26 (86.7) ^b	9 (75.0)	
Missing data, n (%)	/	/	/	/	/	/	/
Anti-herpesvirus, n (%)							
Ganciclovir	6 (2.8)	13 (12.5) ^a	9 (10.6)	5 (7.5)	1 (3.3)	1 (8.3)	0.012
Acyclovir	3 (1.4)	9 (8.7) ^a	4 (4.7)	5 (7.5)	1 (3.3)	1 (8.3)	0.020
Others [§]	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	1 (3.3)	1 (8.3)	0.010
Steroid	105 (49.5)	55 (52.9)	54 (63.5)	26 (38.8)	15 (50.0)	10 (83.3)	0.013
Hospital stays, median (IQR)	27 (15, 46.2)	26 (19.8, 44.2)	27 (18, 54)	27 (18.5, 53)	39.5 (26.2, 58.8)	35 (26.8, 43)	0.160
ICU stays, median (IQR)	13 (7, 26.2)	15 (9, 26.8)	15 (9, 23)	13 (7, 20.5)	18.5 (11, 25)	8.5 (4.8, 13.5)	0.057
Time from ICU admission to mNGS testing, median (IQR)	3 (2, 7)	6 (2.8, 12) ^a	3 (2, 8)	4 (2, 11.5)	4 (3, 8.8)	3 (2, 3.2)	0.003
28-day mortality, n (%)	67 (31.6)	46 (44.2)	36 (42.4)	24 (35.8)	9 (30.0)	6 (50.0)	0.181

Notes:

Pairwise tests were performed for significant variables, with Bonferroni correction to control false positives. In the table, the symbols ^a, ^b, ^c, ^d, and ^e indicate statistical significance between groups, where these symbols represent an adjusted *P* value < 0.05.

^a Compared with non-herpesvirus;

^b Compared with HSV-1;

^c Compared with CMV;

^d Compared with EBV;

^e Compared with HHV-7.

[#] Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

[†] Statistical analysis of clinical metagenomics results were performed among five groups of herpesvirus patients.

[§] Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviations: IQR, interquartile range; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Table S3. Clinical characteristics of different herpesviruses in patients with ventilator-associated pneumonia

Variables	Non-herpesvirus (n=136)	HSV-1 (n=97)	CMV (n=33)	EBV (n=32)	HHV-7 (n=12)	HHV-6B (n=6)	P value
Age, years, median (IQR)	61 (48, 72.2)	66 (51, 73)	66 (59, 73)	68 (55.8, 71.5)	59.5 (42.2, 69.5)	68.5 (60.5, 74.2)	0.203
Male gender, n (%)	95 (69.9)	71 (73.2)	26 (78.8)	20 (62.5)	10 (83.3)	5 (83.3)	0.661
Comorbidities, n (%)							
Diabetes mellitus	31 (22.8)	26 (26.8)	6 (18.2)	8 (25.0)	0 (0.0)	1 (16.7)	0.385
Myocardial infarction	5 (3.7)	9 (9.3)	2 (6.1)	4 (12.5)	2 (16.7)	0 (0.0)	0.156
Chronic pulmonary disease	18 (13.2)	26 (26.8)	10 (30.3)	7 (21.9)	4 (33.3)	3 (50.0)	0.016
Liver disease	8 (5.9)	3 (3.1)	1 (3.0)	1 (3.1)	0 (0.0)	0 (0.0)	0.920
Chronic kidney disease	5 (3.7)	11 (11.3)	5 (15.2)	4 (12.5)	1 (8.3)	0 (0.0)	0.080
Solid tumor	12 (8.8)	15 (15.5)	5 (15.2)	4 (12.5)	1 (8.3)	1 (16.7)	0.580
Hematologic malignancy	1 (0.7)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Connective tissue disease	0 (0.0)	1 (1.0)	0 (0.0)	2 (6.2)	0 (0.0)	0 (0.0)	0.071
Transplantation	3 (2.2)	3 (3.1)	3 (9.1)	1 (3.1)	1 (8.3)	1 (16.7)	0.112
Cerebrovascular disease	25 (18.4)	11 (11.3)	7 (21.2)	3 (9.4)	2 (16.7)	0 (0.0)	0.453
Immunosuppression, n (%)	8 (5.9)	14 (14.4)	5 (15.2)	10 (31.2) ^a	1 (8.3)	3 (50.0)	<0.001
Time from intubation to VAP onset, median (IQR)	4.5 (2, 8)	7 (2, 11)	7 (2, 15)	5 (2, 11.5)	10.5 (7.8, 13.2)	15 (7.5, 15)	0.032
Duration of IMV, median (IQR)	11 (7, 22.5)	14 (7, 21)	17 (11, 27)	12 (7, 17.5)	13.5 (8.2, 18.5)	22 (20.2, 26.8)	0.065
Ventilator-free days at day 28, median (IQR)	4.5 (0, 18)	5 (0, 15)	1 (0, 12)	9.5 (0, 18)	8 (0.8, 15.2)	0 (0, 0)	0.137

Laboratory indicators, median (IQR)							
White blood cell ($\times 10^9/L$)	12.3 (8.7, 16.7)	11.4 (7.6, 16.5)	11.3 (8.8, 17.8)	10.7 (8.7, 14.4)	10.1 (9.0, 13.1)	9.8 (8.3, 10.4)	0.541
Lymphocyte ($\times 10^9/L$)	0.8 (0.5, 1.2)	0.6 (0.3, 1.0)	0.5 (0.3, 0.9)	0.5 (0.4, 0.9)	0.9 (0.5, 1.3)	0.8 (0.5, 1.2)	0.153
Neutrophil ($\times 10^9/L$)	11.1 (7.1, 15.0)	9.8 (6.6, 14.7)	10.5 (7.7, 15.5)	9.5 (6.8, 13.0)	8.9 (5.4, 11.6)	7.7 (6.4, 8.6)	0.401
C-reactive protein (mg/L)	49.3 (10.6, 131.7)	55.9 (12.2, 123.2)	69.8 (22.7, 131.9)	40.5 (11.2, 121.2)	75.5 (30.9, 116.0)	38.2 (17.6, 52.5)	0.879
Procalcitonin (ng/ml)	0.6 (0.2, 5.4)	0.8 (0.2, 3.4)	1.1 (0.4, 7.5)	0.7 (0.2, 8.3)	0.6 (0.3, 1.6)	0.6 (0.2, 2.5)	0.612
SOFA at the time of ICU admission, median (IQR)	7 (4, 9)	7 (5, 9)	8 (6, 11)	6.5 (5, 8)	7 (5.5, 8.2)	7.0 (5, 9)	0.354
SOFA at the time of mNGS testing, median (IQR)	8 (5, 10)	7 (5, 10)	8 (5, 10)	6 (5, 9.2)	6.5 (4.5, 7.5)	8 (4.8, 9)	0.700
Organ dysfunction at the time of mNGS testing, n (%)							
Respiratory	104 (76.5)	78 (80.4)	31 (93.9)	24 (75.0)	10 (83.3)	4 (66.7)	0.207
Coagulation	56 (41.2)	34 (35.1)	12 (36.4)	10 (31.2)	3 (25.0)	1 (16.7)	0.696
Hepatic	29 (21.3)	19 (19.6)	3 (9.1)	5 (15.6)	2 (16.7)	1 (16.7)	0.719
Cardiovascular	57 (41.9)	45 (46.4)	15 (45.5)	11 (34.4)	4 (33.3)	3 (50.0)	0.829
Neurologic	73 (53.7)	44 (45.4)	13 (39.4)	10 (31.2)	4 (33.3)	2 (33.3)	0.169
Kidney	26 (19.1)	22 (22.7)	11 (33.3)	7 (21.9)	0 (0.0)	2 (33.3)	0.172
Results of clinical metagenomics [†]							
Reads, median (IQR)	/	505.0 (41.8, 9362.5)	47.0 (6.0, 143.0) ^b	50.5 (12.5, 175.8) ^b	7.5 (3.0, 61.3) ^b	40.5 (7.8, 182.8)	<0.001
≥ 100 (Reactivation), n (%)	/	60 (62.5)	11 (33.3)	12 (37.5)	3 (25.0)	2 (33.3)	0.004
<100 (Detection), n (%)	/	36 (37.5)	22 (66.7)	20 (62.5)	9 (75.0)	4 (66.7)	

Missing data, n (%)	/	1 (1.0)	/	/	/	/	
Anti-herpesvirus, n (%)							
Ganciclovir	4 (2.9)	5 (5.2)	3 (9.1)	4 (12.5)	3 (25.0)	0 (0.0)	0.025
Acyclovir	2 (1.5)	7 (7.2)	1 (3.0)	1 (3.1)	0 (0.0)	1 (16.7)	0.110
Others [§]	1 (0.7)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Steroid	56 (41.2)	52 (53.6)	18 (54.5)	16 (50.0)	6 (50.0)	4 (66.7)	0.388
Hospital stays, median (IQR)	24 (13, 41.2)	30 (16, 46)	38 (20, 58)	23.5 (14, 47)	34 (17.5, 59)	22 (15.8, 29.8)	0.259
ICU stays, median (IQR)	18 (9, 31)	20 (11, 28)	25 (14, 50)	16 (11.8, 24.5)	17.5 (14, 24.5)	20.5 (15.5, 28.5)	0.189
Time from ICU admission to mNGS testing, median (IQR)	6 (3, 10)	9 (5, 13)	9 (3, 16)	7 (3.8, 12.5)	11.5 (9.8, 14.2)	16 (8.5, 16)	0.012
28-day mortality, n (%)	49 (36.0)	35 (36.1)	10 (30.3)	13 (40.6)	5 (41.7)	4 (66.7)	0.665

Notes:

Pairwise tests were performed for significant variables, with Bonferroni correction to control false positives. In the table, the symbols ^a, ^b, ^c, ^d, and ^e indicate statistical significance between groups, where these symbols represent an adjusted *P* value < 0.05.

^a Compared with non-herpesvirus;

^b Compared with HSV-1;

^c Compared with CMV;

^d Compared with EBV;

^e Compared with HHV-7.

[#] Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

[†] Statistical analysis of clinical metagenomics results were performed among five groups of herpesvirus patients, with one case excluded due to missing reads data.

[§] Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviations: IQR, interquartile range; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Table S4. Clinical characteristics of different herpesviruses in severe pneumonia patients treated with anti-herpesvirus therapy

Variables	Non-herpesvirus (n=28)	HSV-1 (n=73)	CMV (n=56)	EBV (n=35)	HHV-7 (n=7)	HHV-6B (n=5)	<i>P</i> value
Age, years, median (IQR)	59 (53.5, 69.5)	66 (56, 73)	65 (49.2, 70)	66 (58.5, 70)	40 (36, 47)	52 (37, 67)	0.067
Male gender, n (%)	19 (67.9)	51 (69.9)	36 (64.3)	23 (65.7)	6 (85.7)	3 (60.0)	0.902
Comorbidities, n (%)							
Diabetes mellitus	5 (17.9)	17 (23.3)	15 (26.8)	6 (17.1)	0 (0.0)	0 (0.0)	0.557
Myocardial infarction	1 (3.6)	3 (4.1)	1 (1.8)	2 (5.7)	1 (14.3)	0 (0.0)	0.530
Chronic pulmonary disease	9 (32.1)	18 (24.7)	11 (19.6)	10 (28.6)	2 (28.6)	1 (20.0)	0.830
Liver disease	0 (0.0)	6 (8.2)	7 (12.5)	3 (8.6)	0 (0.0)	0 (0.0)	0.465
Chronic kidney disease	2 (7.1)	12 (16.4)	17 (30.4)	10 (28.6)	2 (28.6)	0 (0.0)	0.070
Solid tumor	1 (3.6)	11 (15.1)	11 (19.6)	7 (20.0)	0 (0.0)	0 (0.0)	0.286
Hematologic malignancy	4 (14.3)	4 (5.5)	4 (7.1)	2 (5.7)	0 (0.0)	2 (40.0)	0.133
Connective tissue disease	1 (3.6)	2 (2.7)	6 (10.7)	5 (14.3)	0 (0.0)	2 (40.0)	0.028
Transplantation	5 (17.9)	4 (5.5)	10 (17.9)	3 (8.6)	1 (14.3)	1 (20.0)	0.143
Cerebrovascular disease	3 (10.7)	11 (15.1)	5 (8.9)	5 (14.3)	0 (0.0)	1 (20.0)	0.762
Immunosuppression, n (%)	13 (46.4)	27 (37.0)	32 (57.1)	18 (51.4)	1 (14.3)	5 (100.0)	0.013
Type of pneumonia, n (%)							0.277
CAP	15 (53.6)	41 (56.2)	40 (71.4)	22 (62.9)	2 (28.6)	2 (40.0)	
HAP	7 (25.0)	20 (27.4)	12 (21.4)	8 (22.9)	2 (28.6)	2 (40.0)	
VAP	6 (21.4)	12 (16.4)	4 (7.1)	5 (14.3)	3 (42.9)	1 (20.0)	
Respiratory support, n (%)							0.147
IMV	27 (96.4)	62 (84.9)	49 (87.5)	29 (82.9)	4 (57.1)	5 (100.0)	

NIV	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Others [#]	1 (3.6)	11 (15.1)	7 (12.5)	6 (17.1)	3 (42.9)	0 (0.0)	
Duration of IMV, median (IQR)	9.5 (5, 24.2)	8 (4, 19)	8 (3.8, 14.2)	7 (2.5, 13.5)	6 (0, 12.5)	6 (3, 14)	0.486
Ventilator-free days at day 28, median (IQR)	1 (0, 20.5)	1 (0, 13)	1 (0, 17.2)	2 (0, 19.5)	1 (0, 10.5)	22 (14, 25)	0.414
Laboratory indicators, median (IQR)							
White blood cell ($\times 10^9/L$)	12.1 (5.8, 15.1)	11.3 (6.0, 16.3)	11.1 (6.2, 15.8)	11.0 (4.9, 13.8)	8.5 (7.9, 11.9)	7.5 (5.2, 8.5)	0.718
Lymphocyte ($\times 10^9/L$)	0.7 (0.4, 1.0)	0.4 (0.2, 0.8)	0.4 (0.2, 0.6) ^a	0.3 (0.2, 0.6)	0.7 (0.3, 0.8)	0.3 (0.2, 0.6)	0.036
Neutrophil ($\times 10^9/L$)	11.1 (4.4, 13.9)	9.3 (4.7, 14.6)	10.6 (6.0, 14.2)	10.4 (4.3, 12.8)	7.3 (6.4, 11.4)	6.2 (3.4, 8.2)	0.746
C-reactive protein (mg/L)	102.0 (14.0, 202.8)	69.5 (22.7, 161.2)	82.4 (43.6, 149.3)	89.0 (34.3, 160.0)	29.1 (9.4, 68.9)	54.9 (35.7, 56.8)	0.354
Procalcitonin (ng/ml)	0.8 (0.2, 5.6)	0.6 (0.2, 3.1)	1.0 (0.3, 5.3)	1.1 (0.2, 3.1)	0.2 (0.2, 1.4)	0.2 (0.2, 5.5)	0.630
SOFA at ICU admission, median (IQR)	6 (5, 8.2)	7 (5, 9)	6.5 (5, 10)	7 (4, 8)	4 (4, 5.5)	4 (3, 5)	0.356
SOFA at mNGS testing, median (IQR)	6 (5, 8.2)	7 (4, 9)	8 (6, 11)	7 (5.5, 9)	6 (3.5, 7)	7 (4, 12)	0.178
Organ dysfunction at the time of mNGS testing, n (%)							
Respiratory	23 (82.1)	58 (79.5)	51 (91.1)	31 (88.6)	5 (71.4)	4 (80.0)	0.317
Coagulation	6 (21.4)	33 (45.2)	25 (44.6)	16 (45.7)	2 (28.6)	2 (40.0)	0.284
Hepatic	3 (10.7)	7 (9.6)	7 (12.5)	6 (17.1)	1 (14.3)	0 (0.0)	0.844
Cardiovascular	14 (50.0)	36 (49.3)	34 (60.7)	15 (42.9)	2 (28.6)	3 (60.0)	0.454

Neurologic	4 (14.3)	20 (27.4)	13 (23.2)	7 (20.0)	1 (14.3)	1 (20.0)	0.820
Kidney	5 (17.9)	13 (17.8)	17 (30.4)	7 (20.0)	2 (28.6)	1 (20.0)	0.582
Results of clinical metagenomics [†]							
Reads, median (IQR)	/	8132.0 (558.0, 63589.0)	65.0 (13.0, 411.0) ^b	58.0 (7.0, 406.0) ^b	13.0 (6.5, 92.0) ^b	8.0 (7.0, 62.0) ^b	<0.001
≥100 (Reactivation), n (%)	/	60 (82.2)	23 (41.1) ^b	14 (40.0) ^b	2 (28.6)	1 (20.0)	<0.001
<100 (Detection), n (%)	/	13 (17.8)	33 (58.9) ^b	21 (60.0) ^b	5 (71.4)	4 (80.0)	
Missing data, n (%)	/	/	/	/	/	/	
Anti-herpesvirus, n (%)							
Ganciclovir	20 (71.4)	37 (50.7)	46 (82.1) ^b	25 (71.4)	5 (71.4)	3 (60.0)	0.007
Acyclovir	9 (32.1)	39 (53.4)	11 (19.6) ^b	12 (34.3)	2 (28.6)	2 (40.0)	0.004
Others [§]	2 (7.1)	4 (5.5)	0 (0.0)	0 (0.0)	1 (14.3)	1 (20.0)	0.026
Steroid	23 (82.1)	47 (64.4)	45 (80.4)	24 (68.6)	5 (71.4)	5 (100.0)	0.190
Hospital stays, median (IQR)	27 (16.2, 57)	23 (16, 40)	19.5 (10.8, 39)	23 (12.5, 31)	30 (19, 66)	30 (27, 42)	0.160
ICU stays, median (IQR)	15.5 (9.8, 28.5)	15 (8, 25)	13.5 (8, 21)	12 (8, 18)	20 (15.5, 26.5)	12 (7, 20)	0.404
Time from ICU admission to mNGS testing, median (IQR)	3 (2, 5.5)	3 (2, 11)	2 (2, 4) ^b	3 (2, 5.5)	3 (2.5, 7)	1 (1, 1)	0.006
28-day mortality, n (%)	11 (39.3)	28 (38.4)	24 (42.9)	13 (37.1)	2 (28.6)	1 (20.0)	0.950

Notes:

Pairwise tests were performed for significant variables, with Bonferroni correction to control false positives. In the table, the symbols ^a, ^b, ^c, ^d, and ^e indicate statistical significance between groups, where these symbols represent an adjusted *P* value < 0.05.

^a Compared with non-herpesvirus;

^b Compared with HSV-1;

^c Compared with CMV;

^d Compared with EBV;

^e Compared with HHV-7.

[#] Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

[†] Statistical analysis of clinical metagenomics results was performed among five groups of herpesvirus patients.

[§] Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviations: IQR, interquartile range; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Table S5. Clinical characteristics of different herpesviruses in severe pneumonia patients treated without anti-herpesvirus therapy

Variables	Non-herpesvirus (n=881)	HSV-1 (n=393)	CMV (n=253)	EBV (n=254)	HHV-7 (n=85)	HHV-6B (n=34)	<i>P</i> value
Age, years, median (IQR)	66 (54, 75)	67 (57, 76)	68 (57, 75)	68.5 (59.2, 77)	64 (51, 73) ^d	58.5 (49, 75.2)	0.010
Male gender, n (%)	606 (68.8)	280 (71.2)	187 (73.9)	180 (70.9)	68 (80.0)	22 (64.7)	0.219
Comorbidities, n (%)							
Diabetes mellitus	234 (26.6)	91 (23.2)	58 (22.9)	61 (24.0)	15 (17.6)	8 (23.5)	0.424
Myocardial infarction	62 (7.0)	25 (6.4)	15 (5.9)	12 (4.7)	4 (4.7)	2 (5.9)	0.805
Chronic pulmonary disease	162 (18.4)	90 (22.9)	63 (24.9)	50 (19.7)	13 (15.3)	7 (20.6)	0.139
Liver disease	48 (5.4)	36 (9.2)	25 (9.9)	19 (7.5)	3 (3.5)	0 (0.0)	0.018
Chronic kidney disease	96 (10.9)	51 (13.0)	48 (19.0) ^a	44 (17.3)	12 (14.1)	1 (2.9)	0.003
Solid tumor	125 (14.2)	58 (14.8)	42 (16.6)	41 (16.1)	14 (16.5)	3 (8.8)	0.791
Hematologic malignancy	37 (4.2)	14 (3.6)	22 (8.7)	11 (4.3)	4 (4.7)	4 (11.8)	0.015
Connective tissue disease	30 (3.4)	12 (3.1)	17 (6.7)	21 (8.3) ^a	4 (4.7)	3 (8.8)	0.005
Transplantation	29 (3.3)	15 (3.8)	21 (8.3) ^a	22 (8.7) ^a	3 (3.5)	2 (5.9)	0.001
Cerebrovascular disease	160 (18.2)	57 (14.5)	43 (17.0)	37 (14.6)	17 (20.0)	6 (17.6)	0.522
Immunosuppression, n (%)	166 (18.8)	92 (23.4)	96 (37.9) ^{ab}	92 (36.2) ^{ab}	26 (30.6)	15 (44.1) ^a	<0.001
Type of pneumonia, n (%)							0.002
CAP	546 (62.0)	224 (57.0)	151 (59.7) ^b	168 (66.1) ^b	48 (56.5)	19 (55.9)	
HAP	205 (23.3)	84 (21.4)	73 (28.9) ^b	59 (23.2) ^b	28 (32.9)	10 (29.4)	
VAP	130 (14.8)	85 (21.6)	29 (11.5) ^b	27 (10.6) ^b	9 (10.6)	5 (14.7)	

Respiratory support, n (%)							<0.001
IMV	781 (88.6)	336 (85.5)	218 (86.2)	202 (79.5) ^a	65 (76.5)	24 (70.6) ^a	
NIV	26 (3.0)	12 (3.1)	11 (4.3)	12 (4.7) ^a	5 (5.9)	4 (11.8) ^a	
Others [#]	74 (8.4)	45 (11.5)	24 (9.5)	40 (15.7) ^a	15 (17.6)	6 (17.6) ^a	
Duration of IMV, median (IQR)	8 (4, 16)	9 (3, 17)	8 (4, 16)	7 (1, 12.8) ^{ab}	6 (1, 12)	8.5 (0, 22.2)	0.004
Ventilator-free days at day 28, median (IQR)	1 (0, 17)	1 (0, 14)	1 (0, 13)	1 (0, 14.8)	1 (0, 18)	0 (0, 1) ^a	0.003
Laboratory indicators, median (IQR)							
White blood cell ($\times 10^9/L$)	10.9 (6.7, 15.6)	11.4 (7.6, 16.5)	10.7 (6.9, 15.3)	10.8 (7.1, 15.5)	10.6 (7.3, 14.3)	10.0 (7.5, 15.3)	0.641
Lymphocyte ($\times 10^9/L$)	0.6 (0.3, 1.0)	0.6 (0.3, 1.0)	0.5 (0.3, 0.8)	0.5 (0.3, 1.0)	0.7 (0.3, 1.0)	0.6 (0.3, 1.2)	0.054
Neutrophil ($\times 10^9/L$)	9.4 (5.5, 13.9)	9.8 (6.6, 14.7)	9.3 (5.8, 13.7)	9.3 (5.8, 13.5)	9.4 (5.8, 12.8)	8.3 (6.3, 12.6)	0.464
C-reactive protein (mg/L)	89.4 (31.1, 165.4)	81.9 (28.4, 149.6)	88.7 (36.5, 148.0)	94.8 (37.0, 143.8)	103.4 (39.7, 145.2)	84.7 (26.5, 193.3)	0.903
Procalcitonin (ng/ml)	1.0 (0.2, 8.0)	0.9 (0.3, 3.5)	0.9 (0.3, 4.0)	0.9 (0.3, 4.5)	0.7 (0.3, 3.3)	0.8 (0.1, 1.8)	0.759
SOFA at the time of ICU admission, median (IQR)	7 (4, 9)	6 (4, 9)	7 (4, 9)	7 (4, 9)	6 (4, 8)	6 (4, 9)	0.245
SOFA at the time of mNGS testing, median (IQR)	8 (5, 10)	7 (5, 10)	7 (5, 10)	7 (5, 11)	7 (4, 9)	6.5 (4, 8.8)	0.053
Organ dysfunction at the time of mNGS testing, n (%)							
Respiratory	750 (85.1)	326 (83.0)	216 (85.4)	212 (83.5)	63 (74.1)	25 (73.5)	0.066
Coagulation	295 (33.5)	152 (38.7)	98 (38.7)	92 (36.2)	31 (36.5)	13 (38.2)	0.475
Hepatic	135 (15.3)	76 (19.3)	41 (16.2)	36 (14.2)	11 (12.9)	3 (8.8)	0.296

Cardiovascular	462 (52.4)	187 (47.6)	127 (50.2)	116 (45.7)	27 (31.8) ^a	11 (32.4)	0.001
Neurologic	347 (39.4)	155 (39.4)	83 (32.8)	83 (32.7)	34 (40.0)	13 (38.2)	0.215
Kidney	196 (22.2)	84 (21.4)	69 (27.3)	70 (27.6)	14 (16.5)	8 (23.5)	0.135
Results of clinical metagenomics†							
Reads, median (IQR)	/	119.5 (14.0,3076.0)	23.0 (6.0,147.0) ^b	25.5 (5.3,125.3) ^b	8.0 (4.0,35.0) ^{bcd}	20.0 (5.0,82.3) ^b	<0.001
≥100 (Reactivation), n (%)	/	201 (51.3)	73 (29.0) ^b	69 (27.2) ^b	10 (11.8) ^{bc}	8 (23.5) ^b	<0.001
<100 (Detection), n (%)	/	191 (48.7)	179 (71.0) ^b	185 (72.8) ^b	75 (88.2) ^{bc}	26 (76.5) ^b	
Missing data, n (%)	/	1 (0.3)	1 (0.4)	/	/	/	/
Steroid	473 (53.7)	205 (52.2)	165 (65.2) ^{ab}	134 (52.8)	46 (54.1)	21 (61.8)	0.017
Hospital stays, median (IQR)	20 (12, 33)	23 (13, 37)	22 (12, 41)	20.5 (11, 35)	27 (13, 41)	26.5 (11.8, 39.8)	0.076
ICU stay, median (IQR)	13 (7, 23)	15 (8, 26)	13 (7, 23)	11 (7, 22) ^b	12 (8, 24)	11 (8, 20.8)	0.048
Time from ICU admission to mNGS testing, median (IQR)	3 (2, 6)	4 (3, 10) ^a	3 (2, 7) ^{ab}	3 (2, 8) ^a	4 (2, 8)	3 (3, 6)	<0.001
28-day mortality, n (%)	347 (39.4)	173 (44.0)	120 (47.4)	117 (46.1)	33 (38.8)	19 (55.9)	0.059

Notes:

Pairwise tests were performed for significant variables, with Bonferroni correction to control false positives. In the table, the symbols ^a, ^b, ^c, ^d, and ^e indicate statistical significance between groups, where these symbols represent an adjusted *P* value < 0.05.

^a Compared with non-herpesvirus;

^b Compared with HSV-1;

^c Compared with CMV;

^d Compared with EBV;

^e Compared with HHV-7

[#] Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

[†] Statistical analysis of clinical metagenomics results was performed among five groups of herpesvirus patients, with two cases excluded due to missing reads data.

Abbreviations: IQR, interquartile range; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Table S6. Propensity score matching of patients with **HSV-1** and without herpesvirus

Variables	Original cohort					Matched cohort				
	Non-herpesvirus (n=909)	HSV-1 Positive			SMD	Non-herpesvirus (n=383)	HSV-1 Positive			SMD
		Total* (n=466)	Reactivation n (n=261)	Detection (n=212)			Total* (n=383)	Reactivation (n=219)	Detection (n=163)	
Age, years, median (IQR)	66 (54, 75)	67 (56, 75.8)	69 (58, 77)	65.5 (55, 73)	0.096	66 (53, 75)	67 (56.5, 76)	69 (58, 78)	66 (56, 72.5)	0.109
Male gender, n (%)	625 (68.8)	331 (71.0)	180 (69.0)	150 (73.5)	0.050	272 (71.0)	274 (71.5)	152 (69.4)	121 (74.2)	0.012
Comorbidity, n (%)										
Diabetes mellitus	239 (26.3)	108 (23.2)	58 (22.2)	50 (24.5)	0.072	93 (24.3)	81 (21.1)	41 (18.7)	40 (24.5)	0.075
Myocardial infarction	63 (6.9)	28 (6.0)	16 (6.1)	12 (5.9)	0.037	33 (8.6)	24 (6.3)	13 (5.9)	11 (6.7)	0.090
Chronic pulmonary disease	171 (18.8)	108 (23.2)	52 (19.9)	55 (27.0)	0.107	95 (24.8)	87 (22.7)	39 (17.8)	47 (28.8)	0.049
Liver disease	48 (5.3)	42 (9.0)	21 (8.0)	21 (10.3)	0.145	35 (9.1)	27 (7.0)	15 (6.8)	12 (7.4)	0.077
Chronic kidney disease	98 (10.8)	63 (13.5)	36 (13.8)	27 (13.2)	0.084	54 (14.1)	49 (12.8)	28 (12.8)	21 (12.9)	0.038
Solid tumor	126 (13.9)	69 (14.8)	36 (13.8)	33 (16.2)	0.027	59 (15.4)	60 (15.7)	33 (15.1)	27 (16.6)	0.007
Hematologic malignancy	41 (4.5)	18 (3.9)	7 (2.7)	11 (5.4)	0.032	17 (4.4)	16 (4.2)	6 (2.7)	10 (6.1)	0.013
Connective tissue disease	31 (3.4)	14 (3.0)	6 (2.3)	8 (3.9)	0.023	12 (3.1)	13 (3.4)	5 (2.3)	8 (4.9)	0.015
Transplantation	34 (3.7)	19 (4.1)	9 (3.4)	10 (4.9)	0.017	20 (5.2)	14 (3.7)	7 (3.2)	7 (4.3)	0.076
Cerebrovascular disease	163 (17.9)	68 (14.6)	36 (13.8)	32 (15.7)	0.091	70 (18.3)	57 (14.9)	31 (14.2)	26 (16.0)	0.091
Immunosuppression, n (%)	179 (19.7)	119 (25.5)	63 (24.1)	56 (27.5)	0.140	95 (24.8)	91 (23.8)	46 (21.0)	45 (27.6)	0.024
Type of pneumonia, n (%)					0.154					0.032

CAP	561 (61.7)	265 (56.9)	145 (55.6)	120 (58.8)		221 (57.7)	215 (56.1)	123 (56.2)	92 (56.4)	
HAP	212 (23.3)	104 (22.3)	56 (21.5)	48 (23.5)		87 (22.7)	91 (23.8)	50 (22.8)	41 (25.2)	
VAP	136 (15.0)	97 (20.8)	60 (23.0)	36 (17.6)		75 (19.6)	77 (20.1)	46 (21.0)	30 (18.4)	
Respiratory support, n (%)					0.125					0.049
IMV	808 (88.9)	398 (85.4)	222 (85.1)	175 (85.8)		332 (86.7)	328 (85.6)	184 (84.0)	143 (87.7)	
NIMV	26 (2.9)	12 (2.6)	7 (2.7)	5 (2.5)		9 (2.3)	12 (3.1)	7 (3.2)	5 (3.1)	
Others#	75 (8.3)	56 (12.0)	32 (12.3)	24 (11.8)		42 (11.0)	43 (11.2)	28 (12.8)	15 (9.2)	
IMV, days, median (IQR)	8 (4, 16)	9 (3, 17)	10 (4, 19)	7 (3, 15)		9 (4, 17)	9 (3, 17)	9 (3, 18.5)	8 (3, 16)	
Laboratory test, median (IQR)										
White blood cell (×10 ⁹ /L)	11.0 (6.6, 15.6)	11.4 (7.3, 16.5)	11.9 (7.4, 17.2)	10.7 (7.1, 15.4)	0.069	10.7 (6.5, 15.2)	11.3 (7.3, 16.8)	12.0 (7.5, 17.8)	10.3 (6.1, 15.4)	0.019
Lymphocyte (×10 ⁹ /L)	0.6 (0.3, 1.0)	0.6 (0.3, 1.0)	0.5 (0.3, 0.9)	0.6 (0.3, 1.1)	0.061	0.6 (0.3, 1.0)	0.5 (0.3, 1.0)	0.5 (0.3, 0.9)	0.6 (0.3, 1.2)	0.082
Neutrophil (×10 ⁹ /L)	9.5 (5.5, 13.9)	9.8 (5.9, 14.7)	10.4 (6.3, 15.0)	9.1 (5.3, 14.1)	0.088	9.1 (5.4, 13.2)	9.8 (5.9, 14.7)	10.4 (6.5, 15.6)	8.8 (5.2, 14.2)	0.087
C-reactive protein (mg/L)	90.1 (30.3, 168.4)	81.3 (27.0, 158.5)	70.0 (26.1, 144.7)	88.4 (30.2, 165.4)	0.071	81.0 (26.0, 161.1)	84.5 (29.0, 160.0)	69.8 (28.0, 143.7)	105.8 (36.9, 176.7)	0.034
Procalcitonin (ng/ml)	0.9 (0.2, 7.8)	0.8 (0.3, 3.5)	0.8 (0.3, 3.1)	0.9 (0.3, 4.3)	0.216	0.8 (0.2, 5.4)	0.8 (0.3, 3.5)	0.8 (0.2, 3.1)	0.9 (0.3, 4.8)	0.030
SOFA at the time of mNGS testing, median (IQR)	7 (5, 10)	7 (5, 10)	7 (5, 10)	7 (5, 10)	0.048	7 (5, 10)	7 (5, 10)	7 (5, 10)	7 (5, 10)	0.082
Organ dysfunction at the time of mNGS testing, n (%)										
Respiratory	773 (85.0)	384 (82.4)	215 (82.4)	168 (82.4)	0.071	322 (84.1)	316 (82.5)	184 (84.0)	131 (80.4)	0.042
Coagulation	301 (33.1)	185 (39.7)	104 (39.8)	81 (39.7)	0.137	152 (39.7)	147 (38.4)	88 (40.2)	59 (36.2)	0.027

Hepatic	138 (15.2)	83 (17.8)	47 (18.0)	36 (17.6)	0.071	58 (15.1)	65 (17.0)	40 (18.3)	25 (15.3)	0.050
Cardiovascular	476 (52.4)	223 (47.9)	128 (49.0)	95 (46.6)	0.090	186 (48.6)	184 (48.0)	110 (50.2)	74 (45.4)	0.010
Neurologic	351 (38.6)	175 (37.6)	99 (37.9)	76 (37.3)	0.022	152 (39.7)	138 (36.0)	80 (36.5)	58 (35.6)	0.075
Kidney	201 (22.1)	97 (20.8)	50 (19.2)	47 (23.0)	0.032	89 (23.2)	81 (21.1)	40 (18.3)	41 (25.2)	0.050
Reads, median (IQR)	/	230 (20, 6018)	3276 (644, 28046)	14 (4, 34)		/	273.5 (21.2, 6054)	3138 (634.5, 27865)	14 (4, 37)	
ICU admission to mNGS testing, days, median (IQR)	3 (2, 6)	4 (2, 10)	5 (3, 11)	4 (2, 8.2)	0.416	4 (2, 8)	4 (2, 8)	4 (2, 9)	3 (2, 7)	0.028
Anti-herpesvirus, n (%)										
Ganciclovir	20 (2.2)	37 (7.9)	28 (10.7)	9 (4.4)		10 (2.6)	30 (7.8)	23 (10.5)	7 (4.3)	
Acyclovir	9 (1.0)	39 (8.4)	35 (13.4)	4 (2.0)		4 (1.0)	33 (8.6)	30 (13.7)	3 (1.8)	
Others§	2 (0.2)	4 (0.9)	4 (1.5)	0 (0.0)		0 (0.0)	4 (1.0)	4 (1.8)	0 (0.0)	

Notes: Propensity score matching was performed based on variables with the SMD more than 0.1.

SMD represented the difference between the herpesvirus-negative group and the herpesvirus-positive group.

*One HSV-1 case was excluded from herpesvirus reactivation and detection classification due to missing reads data.

#Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

§ Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviation: SMD, standardized mean difference; SD, standard deviation; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Figure S6

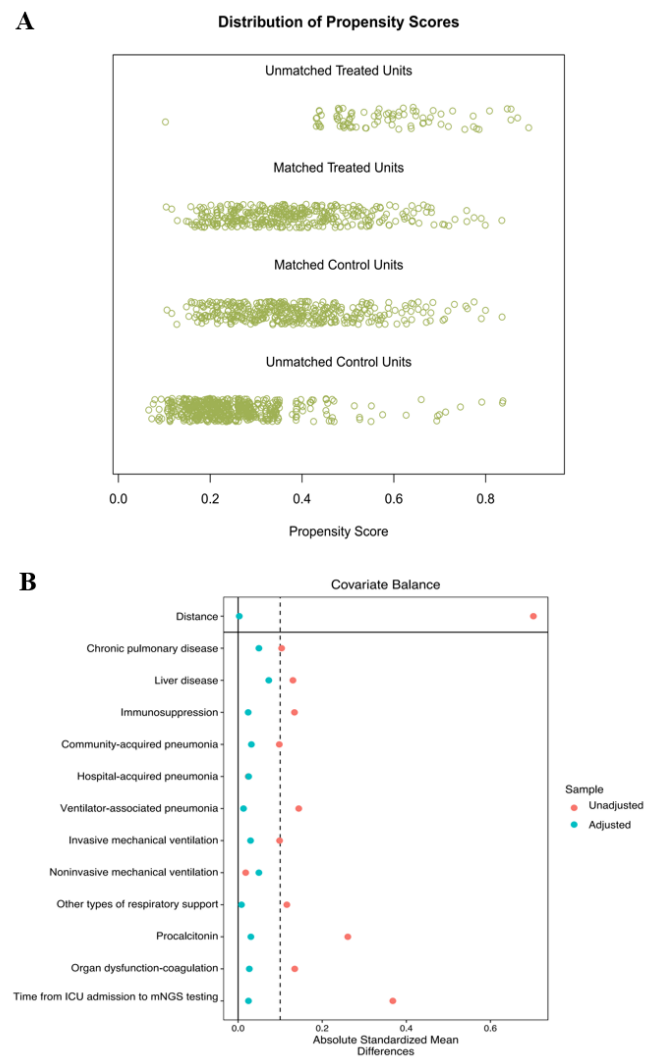


Figure legend:

Figure S6. Covariate pre and post balance plot of HSV-1.

Table S7. Propensity score matching of patients with CMV and without herpesvirus

Variables	Original cohort					Matched cohort				
	Non-herpesvirus (n=909)	CMV Positive			SMD	Non-herpesvirus (n=246)	CMV Positive			SMD
		Total* (n=309)	Reactivation (n=96)	Detection (n=212)			Total* (n=266)	Reactivation (n=81)	Detection (n=184)	
Age, years, median (IQR)	66 (54, 75)	66 (56, 75)	65 (54, 75)	68 (57.8, 75)	0.057	67 (56, 76)	67 (56, 75)	65 (56, 75)	68 (56.8, 75)	<0.001
Male gender, n (%)	625 (68.8)	223 (72.2)	65 (67.7)	157 (74.1)	0.075	189 (71.1)	195 (73.3)	54 (66.7)	140 (76.1)	0.050
Comorbidity, n (%)										
Diabetes mellitus	239 (26.3)	73 (23.6)	18 (18.8)	54 (25.5)	0.062	77 (28.9)	62 (23.3)	16 (19.8)	45 (24.5)	0.129
Myocardial infarction	63 (6.9)	16 (5.2)	6 (6.2)	10 (4.7)	0.074	19 (7.1)	14 (5.3)	5 (6.2)	9 (4.9)	0.078
Chronic pulmonary disease	171 (18.8)	74 (23.9)	23 (24.0)	51 (24.1)	0.126	53 (19.9)	57 (21.4)	17 (21.0)	40 (21.7)	0.037
Liver disease	48 (5.3)	32 (10.4)	13 (13.5)	18 (8.5)	0.190	24 (9.0)	19 (7.1)	8 (9.9)	10 (5.4)	0.069
Chronic kidney disease	98 (10.8)	65 (21.0)	22 (22.9)	43 (20.3)	0.283	49 (18.4)	44 (16.5)	15 (18.5)	29 (15.8)	0.050
Solid tumor	126 (13.9)	53 (17.2)	11 (11.5)	41 (19.3)	0.091	46 (17.3)	43 (16.2)	8 (9.9)	34 (18.5)	0.030
Hematologic malignancy	41 (4.5)	26 (8.4)	9 (9.4)	17 (8.0)	0.159	23 (8.6)	20 (7.5)	7 (8.6)	13 (7.1)	0.041
Connective tissue disease	31 (3.4)	23 (7.4)	11 (11.5)	12 (5.7)	0.179	15 (5.6)	14 (5.3)	8 (9.9)	6 (3.3)	0.017
Transplantation	34 (3.7)	31 (10.0)	13 (13.5)	18 (8.5)	0.250	22 (8.3)	24 (9.0)	9 (11.1)	15 (8.2)	0.027
Cerebrovascular disease	163 (17.9)	48 (15.5)	14 (14.6)	34 (16.0)	0.064	41 (15.4)	45 (16.9)	12 (14.8)	33 (17.9)	0.041
Immunosuppression, n (%)	179 (19.7)	128 (41.4)	52 (54.2)	76 (35.8)	0.485	86 (32.3)	93 (35.0)	39 (48.1)	54 (29.3)	0.056
Type of pneumonia, n (%)					0.146					0.079
CAP	561 (61.7)	191 (61.8)	59 (61.5)	131 (61.8)		171 (64.3)	161 (60.5)	49 (60.5)	111 (60.3)	

HAP	212 (23.3)	85 (27.5)	26 (27.1)	59 (27.8)		65 (24.4)	73 (27.4)	21 (25.9)	52 (28.3)	
VAP	136 (15.0)	33 (10.7)	11 (11.5)	22 (10.4)		30 (11.3)	32 (12.0)	11 (13.6)	21 (11.4)	
Respiratory support, n (%)					0.076					0.115
IMV	808 (88.9)	267 (86.4)	80 (83.3)	186 (87.7)		242 (91.0)	233 (87.6)	70 (86.4)	162 (88.0)	
NIMV	26 (2.9)	11 (3.6)	6 (6.2)	5 (2.4)		7 (2.6)	8 (3.0)	5 (6.2)	3 (1.6)	
Others#	75 (8.3)	31 (10.0)	10 (10.4)	21 (9.9)		17 (6.4)	25 (9.4)	6 (7.4)	19 (10.3)	
IMV, days, median (IQR)	8 (4, 16)	8 (4, 16)	7 (1, 13)	9 (4, 17)		9 (4, 17)	9 (4, 17)	8 (2, 14)	9 (4, 17)	
Laboratory test, median (IQR)										
White blood cell (×10 ⁹ /L)	11.0 (6.6, 15.6)	10.7 (6.9, 15.4)	10.2 (6.9, 14.6)	11.3 (6.9, 15.6)	0.002	11.9 (7.3, 16.4)	10.7 (6.9, 15.3)	10.4 (6.9, 14.6)	11.3 (6.9, 15.6)	0.123
Lymphocyte (×10 ⁹ /L)	0.6 (0.3, 1.0)	0.4 (0.3, 0.8)	0.4 (0.2, 0.7)	0.5 (0.3, 0.8)	0.078	0.6 (0.3, 1.0)	0.5 (0.3, 0.8)	0.4 (0.3, 0.7)	0.5 (0.3, 0.9)	0.105
Neutrophil (×10 ⁹ /L)	9.5 (5.5, 13.9)	9.4 (5.9, 13.8)	9.0 (6.0, 12.7)	9.9 (5.9, 14.2)	0.117	10.1 (5.8, 14.6)	9.4 (5.8, 13.6)	8.8 (6.0, 12.6)	9.7 (5.7, 13.7)	0.021
C-reactive protein (mg/L)	90.1 (30.3, 168.4)	87.7 (37.2, 148.0)	89.8 (38.6, 151.3)	86.0 (37.1, 147.8)	0.070	80.6 (22.7, 155.0)	89.3 (39.5, 153.3)	87.1 (45.5, 161.2)	90.2 (38.2, 152.9)	0.085
Procalcitonin (ng/ml)	0.9 (0.2, 7.8)	0.9 (0.3, 4.1)	1.0 (0.3, 5.5)	0.8 (0.3, 3.9)	0.260	0.9 (0.2, 4.4)	1.0 (0.3, 5.1)	0.9 (0.3, 6.8)	1.0 (0.3, 4.2)	0.036
SOFA at the time of mNGS testing, median (IQR)	7 (5, 10)	7 (5, 10)	8 (5.8, 11.2)	7 (5, 10)	0.037	7 (5, 10)	7 (5, 10)	8 (5, 11)	7 (5, 10)	0.021
Organ dysfunction at the time of mNGS testing, n (%)										
Respiratory	773 (85.0)	267 (86.4)	85 (88.5)	181 (85.4)	0.039	233 (87.6)	232 (87.2)	71 (87.7)	160 (87.0)	0.011
Coagulation	301 (33.1)	123 (39.8)	46 (47.9)	76 (35.8)	0.139	91 (34.2)	98 (36.8)	38 (46.9)	59 (32.1)	0.055

Hepatic	138 (15.2)	48 (15.5)	19 (19.8)	28 (13.2)	0.010	36 (13.5)	39 (14.7)	13 (16.0)	25 (13.6)	0.032
Cardiovascular	476 (52.4)	161 (52.1)	53 (55.2)	108 (50.9)	0.005	143 (53.8)	136 (51.1)	42 (51.9)	94 (51.1)	0.053
Neurologic	351 (38.6)	96 (31.1)	22 (22.9)	73 (34.4)	0.159	88 (33.1)	91 (34.2)	22 (27.2)	68 (37.0)	0.024
Kidney	201 (22.1)	86 (27.8)	34 (35.4)	51 (24.1)	0.132	72 (27.1)	66 (24.8)	27 (33.3)	38 (20.7)	0.051
Reads, median (IQR)	/	28 (7, 191.5)	486.5 (220.2, 1339.5)	11 (5, 31)		/	32 (7, 685)	713 (193, 3191)	14 (5, 48)	
ICU admission to mNGS testing, days, median (IQR)	3 (2, 6)	3 (2, 7)	3 (2, 7)	3 (2, 6)	0.171	3 (2, 7)	3 (2, 7)	3 (2, 7)	3 (2, 7)	0.016
Anti-herpesvirus, n (%)										
Ganciclovir	20 (2.2)	46 (14.9)	20 (20.8)	26 (12.3)		8 (3.0)	40 (15.1)	18 (22.2)	22 (12.0)	
Acyclovir	9 (1.0)	11 (3.6)	3 (3.1)	8 (3.8)		5 (1.9)	8 (3.0)	1 (1.2)	7 (3.8)	
Others§	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

Notes: Propensity score matching was performed based on variables with the SMD more than 0.1.

SMD represented the difference between the herpesvirus-negative group and the herpesvirus-positive group.

*One CMV case was excluded from herpesvirus reactivation and detection classification due to missing reads data.

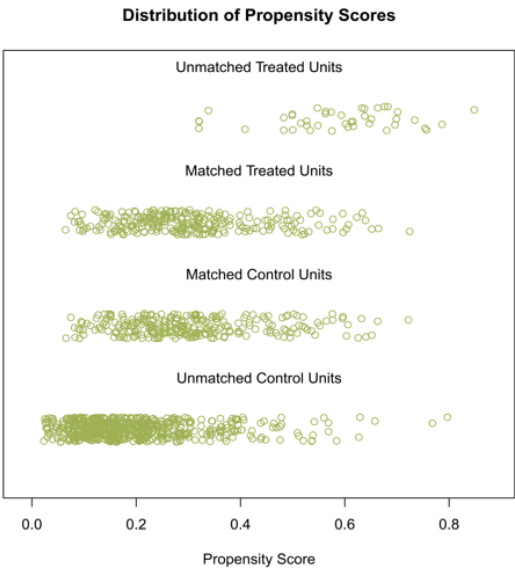
#Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

§ Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviation: SMD, standardized mean difference; SD, standard deviation; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Figure S7

A



B

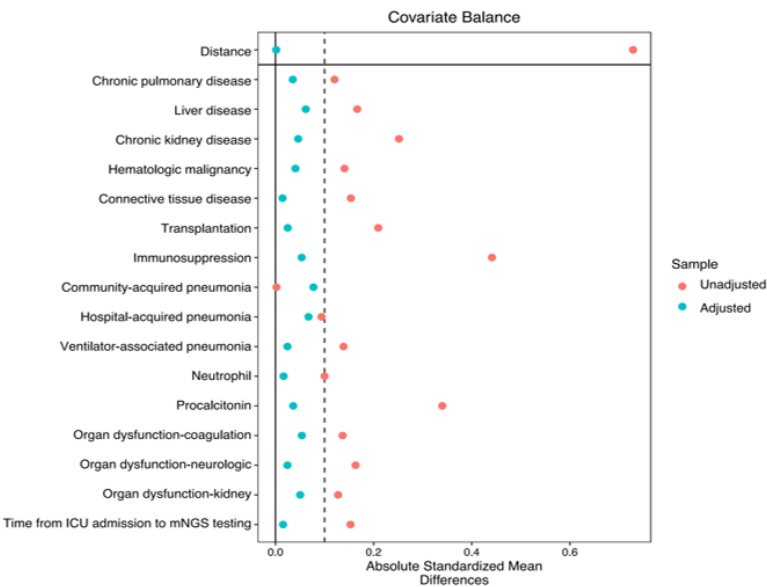


Figure legend:

Figure S7. Covariate pre and post balance plot of CMV.

Table S8. Propensity score matching of patients with EBV and without herpesvirus

Variables	Original cohort					Matched cohort				
	Non-herpesviruses (n=909)	EBV Positive			SMD	Non-herpesviruses (n=246)	EBV Positive			SMD
		Total (n=289)	Reactivation n (n=83)	Detection (n=206)			Total (n=246)	Reactivation n (n=69)	Detection (n=177)	
Age, years, median (IQR)	66 (54, 75)	68 (59, 76)	67 (56, 76.5)	68 (61, 76)	0.174	67 (58, 77)	68 (59, 76)	67 (56, 77)	68 (60, 76)	0.060
Male gender, n (%)	625 (68.8)	203 (70.2)	60 (72.3)	143 (69.4)	0.032	166 (67.5)	173 (70.3)	50 (72.5)	123 (69.5)	0.062
Comorbidity, n (%)										
Diabetes mellitus	239 (26.3)	67 (23.2)	15 (18.1)	52 (25.2)	0.072	76 (30.9)	53 (21.5)	12 (17.4)	41 (23.2)	0.214
Myocardial infarction	63 (6.9)	14 (4.8)	4 (4.8)	10 (4.9)	0.089	24 (9.8)	13 (5.3)	4 (5.8)	9 (5.1)	0.170
Chronic pulmonary disease	171 (18.8)	60 (20.8)	16 (19.3)	44 (21.4)	0.049	47 (19.1)	51 (20.7)	13 (18.8)	38 (21.5)	0.041
Liver disease	48 (5.3)	22 (7.6)	9 (10.8)	13 (6.3)	0.095	13 (5.3)	16 (6.5)	9 (13.0)	7 (4.0)	0.052
Chronic kidney disease	98 (10.8)	54 (18.7)	20 (24.1)	34 (16.5)	0.224	44 (17.9)	35 (14.2)	12 (17.4)	23 (13.0)	0.100
Solid tumor	126 (13.9)	48 (16.6)	15 (18.1)	33 (16.0)	0.077	36 (14.6)	39 (15.9)	12 (17.4)	27 (15.3)	0.034
Hematologic malignancy	41 (4.5)	13 (4.5)	6 (7.2)	7 (3.4)	0.001	17 (6.9)	12 (4.9)	5 (7.2)	7 (4.0)	0.086
Connective tissue disease	31 (3.4)	26 (9.0)	7 (8.4)	19 (9.2)	0.233	19 (7.7)	13 (5.3)	6 (8.7)	7 (4.0)	0.099
Transplantation	34 (3.7)	25 (8.7)	7 (8.4)	18 (8.7)	0.205	16 (6.5)	15 (6.1)	2 (2.9)	13 (7.3)	0.017
Cerebrovascular disease	163 (17.9)	42 (14.5)	13 (15.7)	29 (14.1)	0.092	49 (19.9)	39 (15.9)	13 (18.8)	26 (14.7)	0.106
Immunosuppression, n (%)	179 (19.7)	110 (38.1)	39 (47.0)	71 (34.5)	0.414	73 (29.7)	76 (30.9)	27 (39.1)	49 (27.7)	0.027
Type of pneumonia, n (%)					0.119					0.043

CAP	561 (61.7)	190 (65.7)	53 (63.9)	137 (66.5)		161 (65.4)	156 (63.4)	42 (60.9)	114 (64.4)	
HAP	212 (23.3)	67 (23.2)	18 (21.7)	49 (23.8)		59 (24.0)	62 (25.2)	16 (23.2)	46 (26.0)	
VAP	136 (15.0)	32 (11.1)	12 (14.5)	20 (9.7)		26 (10.6)	28 (11.4)	11 (15.9)	17 (9.6)	
Respiratory support, n (%)					0.253					0.046
IMV	808 (88.9)	231 (79.9)	61 (73.5)	170 (82.5)		202 (82.1)	202 (82.1)	54 (78.3)	148 (83.6)	
NIMV	26 (2.9)	12 (4.2)	4 (4.8)	8 (3.9)		9 (3.7)	11 (4.5)	3 (4.3)	8 (4.5)	
Others#	75 (8.3)	46 (15.9)	18 (21.7)	28 (13.6)		35 (14.2)	33 (13.4)	12 (17.4)	21 (11.9)	
IMV, days, median (IQR)	8 (4, 16)	7 (1, 13)	5 (0, 10)	8 (2, 13.8)		8 (3, 17)	7 (2, 13)	5 (1, 10)	8 (2, 14)	
Laboratory test, median (IQR)										
White blood cell (×10 ⁹ /L)	11.0 (6.6, 15.6)	10.8 (7.0, 14.9)	10.1 (6.6, 13.6)	11.5 (7.4, 15.6)	0.003	10.8 (7.4, 15.0)	10.7 (6.8, 14.8)	10.0 (6.5, 12.9)	11.2 (7.4, 15.5)	0.003
Lymphocyte (×10 ⁹ /L)	0.6 (0.3, 1.0)	0.5 (0.3, 1.0)	0.5 (0.3, 1.0)	0.5 (0.3, 1.0)	0.052	0.6 (0.3, 1.0)	0.5 (0.3, 1.0)	0.6 (0.3, 1.0)	0.5 (0.3, 0.9)	0.063
Neutrophil (×10 ⁹ /L)	9.5 (5.5, 13.9)	9.5 (5.8, 13.3)	8.9 (5.2, 12.3)	10.1 (6.1, 14.1)	0.021	9.6 (6.2, 13.9)	9.3 (5.5, 13.2)	8.1 (4.6, 11.5)	9.9 (5.8, 13.8)	0.057
C-reactive protein (mg/L)	90.1 (30.3, 168.4)	92.6 (35.7, 144.7)	97.1 (29.6, 162.1)	91.8 (37.0, 137.3)	0.070	88.9 (19.3, 162.1)	96.3 (36.0, 145.1)	97.1 (28.3, 161.2)	95.5 (37.8, 141.3)	0.021
Procalcitonin (ng/ml)	0.9 (0.2, 7.8)	0.9 (0.2, 4.4)	0.9 (0.3, 5.7)	0.9 (0.2, 3.7)	0.249	0.8 (0.2, 3.5)	0.8 (0.2, 4.3)	0.8 (0.2, 7.9)	0.8 (0.2, 3.4)	0.078
SOFA at the time of mNGS testing, median (IQR)	7 (5, 10)	7 (5, 10)	7 (5, 9.5)	7 (5, 11)	0.095	7 (4.2, 9.8)	7 (5, 10)	7 (5, 9)	7 (5, 11)	0.057
Organ dysfunction at the time of mNGS testing, n (%)										

Respiratory	773 (85.0)	243 (84.1)	65 (78.3)	178 (86.4)	0.026	206 (83.7)	212 (86.2)	57 (82.6)	155 (87.6)	0.068
Coagulation	301 (33.1)	108 (37.4)	33 (39.8)	75 (36.4)	0.089	82 (33.3)	91 (37.0)	29 (42.0)	62 (35.0)	0.077
Hepatic	138 (15.2)	42 (14.5)	16 (19.3)	26 (12.6)	0.018	26 (10.6)	36 (14.6)	14 (20.3)	22 (12.4)	0.123
Cardiovascular	476 (52.4)	131 (45.3)	30 (36.1)	101 (49.0)	0.141	105 (42.7)	115 (46.7)	23 (33.3)	92 (52.0)	0.082
Neurologic	351 (38.6)	90 (31.1)	24 (28.9)	66 (32.0)	0.157	83 (33.7)	79 (32.1)	20 (29.0)	59 (33.3)	0.035
Kidney	201 (22.1)	77 (26.6)	22 (26.5)	55 (26.7)	0.106	52 (21.1)	54 (22.0)	14 (20.3)	40 (22.6)	0.020
Reads, median (IQR)	/	27 (6, 137)	535 (190.5, 2373.5)	11 (4, 30)		/	34.5 (7.2, 278.5)	267 (93, 7071)	20 (5, 83)	
ICU admission to mNGS testing, days, median (IQR)	3 (2, 6)	3 (2, 7)	3 (2, 8)	3 (2, 7)	0.236	3 (2, 8)	3 (2, 6.8)	3 (2, 7)	3 (2, 6)	0.046
Anti-herpesvirus, n (%)										
Ganciclovir	20 (2.2)	25 (8.7)	9 (10.8)	16 (7.8)		6 (2.4)	21 (8.5)	8 (11.6)	13 (7.3)	
Acyclovir	9 (1.0)	12 (4.2)	6 (7.2)	6 (2.9)		1 (0.4)	10 (4.1)	5 (7.2)	5 (2.8)	
Others§	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)		2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	

Notes: Propensity score matching was performed based on variables with the SMD more than 0.1.

SMD represented the difference between the herpesvirus-negative group and the herpesvirus-positive group.

[#]Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

[§]Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviation: SMD, standardized mean difference; SD, standard deviation; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Figure S8

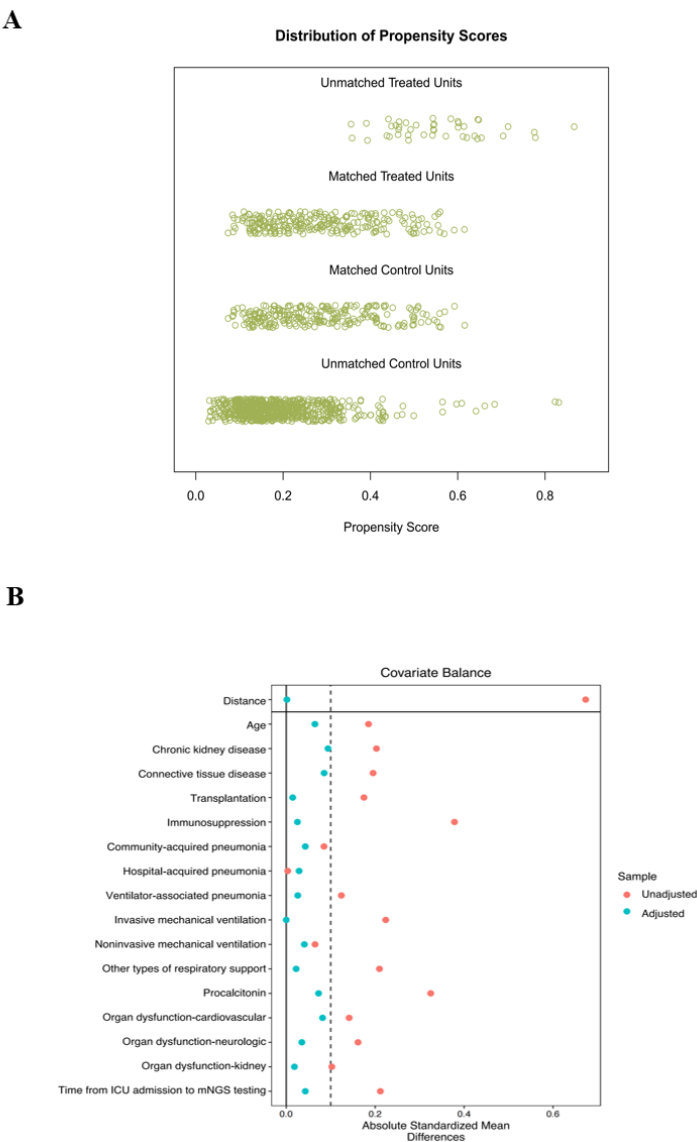


Figure legend:

Figure S8. Covariate pre and post balance plot of EBV.

Table S9. Propensity score matching of patients with HHV-7 and without herpesvirus

Variables	Original cohort					Matched cohort				
	Non-herpesvirus (n=909)	HHV-7 Positive			SMD	Non-herpesvirus (n=73)	HHV-7 Positive			SMD
		Total (n=92)	Reactivation (n=12)	Detection (n=80)			Total (n=73)	Reactivation (n=7)	Detection (n=66)	
Age, years, median (IQR)	66 (54, 75)	61.5 (47.8, 73)	38.5 (25.5, 58.5)	65 (52.5, 73)	0.262	66 (50, 73)	64 (51, 73)	40 (34, 57)	65 (53, 73.8)	0.010
Male gender, n (%)	625 (68.8)	74 (80.4)	9 (75.0)	65 (81.2)	0.271	58 (79.5)	56 (76.7)	5 (71.4)	51 (77.3)	0.066
Comorbidity, n (%)										
Diabetes mellitus	239 (26.3)	15 (16.3)	3 (25.0)	12 (15.0)	0.246	11 (15.1)	14 (19.2)	2 (28.6)	12 (18.2)	0.109
Myocardial infarction	63 (6.9)	5 (5.4)	0 (0.0)	5 (6.2)	0.062	6 (8.2)	4 (5.5)	0 (0.0)	4 (6.1)	0.109
Chronic pulmonary disease	171 (18.8)	15 (16.3)	1 (8.3)	14 (17.5)	0.066	9 (12.3)	11 (15.1)	1 (14.3)	10 (15.2)	0.080
Liver disease	48 (5.3)	3 (3.3)	0 (0.0)	3 (3.8)	0.100	4 (5.5)	3 (4.1)	0 (0.0)	3 (4.5)	0.064
Chronic kidney disease	98 (10.8)	14 (15.2)	2 (16.7)	12 (15.0)	0.132	14 (19.2)	9 (12.3)	1 (14.3)	8 (12.1)	0.189
Solid tumor	126 (13.9)	14 (15.2)	0 (0.0)	14 (17.5)	0.038	10 (13.7)	12 (16.4)	0 (0.0)	12 (18.2)	0.077
Hematologic malignancy	41 (4.5)	4 (4.3)	0 (0.0)	4 (5.0)	0.008	2 (2.7)	3 (4.1)	0 (0.0)	3 (4.5)	0.075
Connective tissue disease	31 (3.4)	4 (4.3)	2 (16.7)	2 (2.5)	0.049	0 (0.0)	3 (4.1)	1 (14.3)	2 (3.0)	0.293
Transplantation	34 (3.7)	4 (4.3)	1 (8.3)	3 (3.8)	0.031	4 (5.5)	3 (4.1)	1 (14.3)	2 (3.0)	0.064
Cerebrovascular disease	163 (17.9)	17 (18.5)	2 (16.7)	15 (18.8)	0.014	8 (11.0)	13 (17.8)	0 (0.0)	13 (19.7)	0.196
Immunosuppression, n (%)	179 (19.7)	27 (29.3)	5 (41.7)	22 (27.5)	0.226	14 (19.2)	19 (26.0)	2 (28.6)	17 (25.8)	0.164
Type of pneumonia, n (%)					0.208					0.166

CAP	561 (61.7)	50 (54.3)	5 (41.7)	45 (56.2)		36 (49.3)	42 (57.5)	4 (57.1)	38 (57.6)	
HAP	212 (23.3)	30 (32.6)	4 (33.3)	26 (32.5)		26 (35.6)	22 (30.1)	1 (14.3)	21 (31.8)	
VAP	136 (15.0)	12 (13.0)	3 (25.0)	9 (11.2)		11 (15.1)	9 (12.3)	2 (28.6)	7 (10.6)	
Respiratory support, n (%)					0.369					0.064
IMV	808 (88.9)	69 (75.0)	8 (66.7)	61 (76.2)		58 (79.5)	59 (80.8)	5 (71.4)	54 (81.8)	
NIMV	26 (2.9)	5 (5.4)	1 (8.3)	4 (5.0)		4 (5.5)	3 (4.1)	0 (0.0)	3 (4.5)	
Others [#]	75 (8.3)	18 (19.6)	3 (25.0)	15 (18.8)		11 (15.1)	11 (15.1)	2 (28.6)	9 (13.6)	
IMV, days, median (IQR)	8 (4, 16)	6 (0.8, 12.2)	5.5 (0, 13.2)	6 (1, 12)		7 (2, 13)	7 (3, 13)	9 (1, 11.5)	7 (4, 12.8)	
Laboratory test, median (IQR)										
White blood cell ($\times 10^9/L$)	11.0 (6.6, 15.6)	10.4 (7.6, 14.0)	10.1 (8.2, 13.4)	10.6 (7.2, 14.0)	0.072	10.6 (6.4, 16.0)	10.8 (7.7, 14.4)	8.8 (7.4, 10.1)	11.3 (8.1, 14.5)	0.052
Lymphocyte ($\times 10^9/L$)	0.6 (0.3, 1.0)	0.7 (0.3, 1.0)	1.0 (0.7, 1.5)	0.6 (0.3, 0.9) ^b	0.068	0.6 (0.4, 1.2)	0.7 (0.3, 1.0)	0.9 (0.4, 1.3)	0.7 (0.3, 0.9)	0.164
Neutrophil ($\times 10^9/L$)	9.5 (5.5, 13.9)	9.3 (5.7, 12.8)	7.6 (6.4, 11.9)	9.6 (5.6, 12.8)	0.073	8.9 (5.6, 14.3)	9.7 (5.8, 13.2)	7.3 (6.6, 8.9)	9.8 (5.6, 13.2)	0.044
C-reactive protein (mg/L)	90.1 (30.3, 168.4)	97.8 (36.3, 142.2)	69.5 (11.8, 114.0)	97.8 (39.6, 149.5)	0.072	78.9 (14.1, 164.1)	100.5 (37.4, 138.8)	101.8 (11.7, 105.8)	97.8 (39.5, 144.6)	0.095
Procalcitonin (ng/ml)	0.9 (0.2, 7.8)	0.6 (0.3, 3.2)	0.2 (0.2, 4.4)	0.7 (0.3, 2.7)	0.253	1.2 (0.2, 5.8)	0.6 (0.2, 3.2)	0.2 (0.1, 1.3)	0.6 (0.3, 3.8)	0.191
SOFA at the time of mNGS testing, median (IQR)	7 (5, 10)	6 (4, 9)	4.5 (3, 6.2)	7 (4, 9)	0.327	6 (5, 9)	7 (4, 9)	5 (3.5, 6.5)	7 (4.2, 9)	0.011
Organ dysfunction at the time of mNGS testing, n (%)										

Respiratory	773 (85.0)	68 (73.9)	9 (75.0)	59 (73.8)	0.278	56 (76.7)	59 (80.8)	7 (100.0)	52 (78.8)	0.101
Coagulation	301 (33.1)	33 (35.9)	4 (33.3)	29 (36.2)	0.058	26 (35.6)	26 (35.6)	2 (28.6)	24 (36.4)	<0.001
Hepatic	138 (15.2)	12 (13.0)	0 (0.0)	12 (15.0)	0.061	12 (16.4)	12 (16.4)	0 (0.0)	12 (18.2)	<0.001
Cardiovascular	476 (52.4)	29 (31.5)	1 (8.3)	28 (35.0)	0.432	25 (34.2)	27 (37.0)	1 (14.3)	26 (39.4)	0.057
Neurologic	351 (38.6)	35 (38.0)	1 (8.3)	34 (42.5)	0.012	30 (41.1)	31 (42.5)	0 (0.0)	31 (47.0)	0.028
Kidney	201 (22.1)	16 (17.4)	2 (16.7)	14 (17.5)	0.119	16 (21.9)	14 (19.2)	1 (14.3)	13 (19.7)	0.068
Reads, median (IQR)	/	8 (3.8, 37.8)	176.5 (146.2, 996.5)	7.0 (3, 17.8)		/	28 (6, 163)	937 (448.5, 2372)	14 (5.2, 119.2)	
ICU admission to mNGS testing, days, median (IQR)	3 (2, 6)	4 (2, 8.2)	8.5 (3.8, 13.2)	3 (2, 7)	0.300	4 (2, 8)	3 (2, 5)	4 (3, 10.5)	3 (2, 5)	0.232
Anti-herpesvirus, n (%)										
Ganciclovir	20 (2.2)	5 (5.4)	1 (8.3)	4 (5.0)		3 (4.1)	4 (5.5)	1 (14.3)	3 (4.5)	
Acyclovir	9 (1.0)	2 (2.2)	1 (8.3)	1 (1.2)		0 (0.0)	1 (1.4)	1 (14.3)	0 (0.0)	
Others [§]	2 (0.2)	1 (1.1)	0 (0.0)	1 (1.2)		0 (0.0)	1 (1.4)	0 (0.0)	1 (1.5)	

Notes: Propensity score matching was performed based on variables with the SMD more than 0.1.

SMD represented the difference between the herpesvirus-negative group and the herpesvirus-positive group.

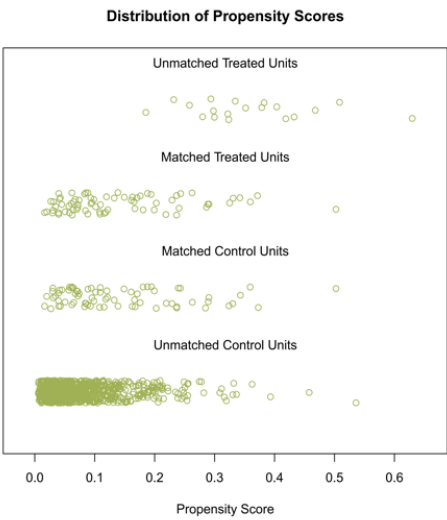
[#]Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

[§]Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviation: SMD, standardized mean difference; SD, standard deviation; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Figure S9

A



B

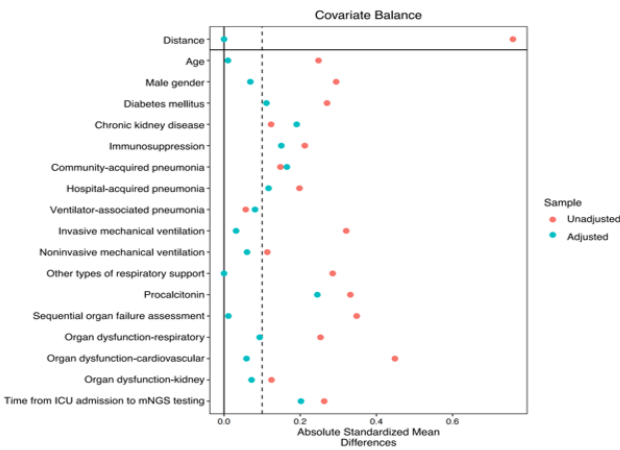


Figure legend:

Figure S9. Covariate pre and post balance plot of HHV-7.

Table S10. Propensity score matching of patients with **HHV-6B** and without herpesvirus

Variables	Original cohort					Matched cohort				
	Non-herpesvirus (n=909)	HHV-6B Positive			SMD	Non-herpesvirus (n=32)	HHV-6B Positive			SMD
		Total (n=39)	Reactivation n (n=9)	Detection (n=30)			Total (n=32)	Reactivation (n=6)	Detection (n=26)	
Age, years, median (IQR)	66 (54, 75)	58 (48.5, 73)	56 (28, 60)	61 (49, 73)	0.268	66.5 (46.8, 73)	59.5 (49, 73.8)	59.5 (56.8, 72)	61 (49, 72)	0.044
Male gender, n (%)	625 (68.8)	25 (64.1)	6 (66.7)	19 (63.3)	0.099	24 (75.0)	21 (65.6)	3 (50.0)	18 (69.2)	0.206
Comorbidity, n (%)										
Diabetes mellitus	239 (26.3)	8 (20.5)	1 (11.1)	7 (23.3)	0.137	9 (28.1)	8 (25.0)	1 (16.7)	7 (26.9)	0.071
Myocardial infarction	63 (6.9)	2 (5.1)	0 (0.0)	2 (6.7)	0.076	1 (3.1)	2 (6.2)	0 (0.0)	2 (7.7)	0.148
Chronic pulmonary disease	171 (18.8)	8 (20.5)	2 (22.2)	6 (20.0)	0.043	6 (18.8)	6 (18.8)	1 (16.7)	5 (19.2)	<0.001
Liver disease	48 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)	0.334	0 (0.0)	0 (0.0)	6 (100.0)	26 (100.0)	<0.001
Chronic kidney disease	98 (10.8)	1 (2.6)	0 (0.0)	1 (3.3)	0.334	1 (3.1)	1 (3.1)	0 (0.0)	1 (3.8)	<0.001
Solid tumor	126 (13.9)	3 (7.7)	2 (22.2)	1 (3.3)	0.200	2 (6.2)	3 (9.4)	2 (33.3)	1 (3.8)	0.117
Hematologic malignancy	41 (4.5)	6 (15.4)	3 (33.3)	3 (10.0)	0.369	6 (18.8)	4 (12.5)	2 (33.3)	2 (7.7)	0.173
Connective tissue disease	31 (3.4)	5 (12.8)	1 (11.1)	4 (13.3)	0.350	3 (9.4)	3 (9.4)	0 (0.0)	3 (11.5)	<0.001
Transplantation	34 (3.7)	3 (7.7)	1 (11.1)	2 (6.7)	0.171	4 (12.5)	3 (9.4)	1 (16.7)	2 (7.7)	0.100
Cerebrovascular disease	163 (17.9)	7 (17.9)	1 (11.1)	6 (20.0)	<0.001	6 (18.8)	5 (15.6)	0 (0.0)	5 (19.2)	0.083
Immunosuppression, n (%)	179 (19.7)	20 (51.3)	4 (44.4)	16 (53.3)	0.699	14 (43.8)	14 (43.8)	2 (33.3)	12 (46.2)	<0.001
Type of pneumonia, n (%)					0.178					0.093

CAP	561 (61.7)	21 (53.8)	4 (44.4)	17 (56.7)		19 (59.4)	18 (56.2)	3 (50.0)	15 (57.7)	
HAP	212 (23.3)	12 (30.8)	3 (33.3)	9 (30.0)		9 (28.1)	9 (28.1)	2 (33.3)	7 (26.9)	
VAP	136 (15.0)	6 (15.4)	2 (22.2)	4 (13.3)		4 (12.5)	5 (15.6)	1 (16.7)	4 (15.4)	
Respiratory support, n (%)					0.398					0.140
IMV	808 (88.9)	29 (74.4)	7 (77.8)	22 (73.3)		25 (78.1)	25 (78.1)	5 (83.3)	20 (76.9)	
NIMV	26 (2.9)	4 (10.3)	1 (11.1)	3 (10.0)		2 (6.2)	3 (9.4)	1 (16.7)	2 (7.7)	
Others#	75 (8.3)	6 (15.4)	1 (11.1)	5 (16.7)		5 (15.6)	4 (12.5)	0 (0.0)	4 (15.4)	
IMV, days, median (IQR)	8 (4, 16)	8 (0.5, 20.5)	8 (1, 23)	8.5 (0.8, 19.8)		7 (1, 17)	11 (3.8, 21.5)	10 (8, 20.2)	11 (3.2, 20.8)	
Laboratory test, median (IQR)										
White blood cell (×10 ⁹ /L)	11.0 (6.6, 15.6)	9.9 (7.3, 14.8)	12.1 (9.3, 14.6)	9.8 (7.1, 14.7)	0.129	9.6 (4.1, 12.2)	10.0 (7.5, 14.7)	12.5 (10.9, 14.1)	9.9 (7.3, 14.7)	0.361
Lymphocyte (×10 ⁹ /L)	0.6 (0.3, 1.0)	0.6 (0.3, 1.1)	0.8 (0.6, 1.1)	0.4 (0.2, 1.1)	0.048	0.5 (0.3, 1.1)	0.5 (0.2, 0.9)	0.8 (0.6, 0.8)	0.4 (0.2, 0.9)	0.011
Neutrophil (×10 ⁹ /L)	9.5 (5.5, 13.9)	8.2 (6.2, 12.6)	8.7 (7.2, 11.8)	7.7 (6.2, 13.9)	0.260	8.4 (2.8, 9.6)	8.3 (6.4, 13.0)	10.2 (8.4, 12.4)	8.1 (6.3, 13.9)	0.383
C-reactive protein (mg/L)	90.1 (30.3, 168.4)	56.8 (26.8, 165.4)	22.7 (10.8, 99.0)	80.7 (36.0, 193.3)	0.066	99.9 (25.5, 170.9)	55.8 (27.1, 147.2)	18.5 (7.0, 79.9)	63.6 (35.7, 165.6)	0.190
Procalcitonin (ng/ml)	0.9 (0.2, 7.8)	0.7 (0.1, 2.5)	1.0 (0.4, 5.9)	0.4 (0.1, 1.8)	0.182	1.3 (0.3, 10.5)	0.3 (0.1, 2.2)	0.9 (0.2, 5.6)	0.3 (0.1, 1.8)	0.299
SOFA at the time of mNGS testing, median (IQR)	7 (5, 10)	7 (4, 9)	5 (4, 8)	7 (4, 9.8)	0.207	7 (4.8, 9.2)	7.5 (4, 10)	6.5 (4.2, 8)	7.5 (4.2, 10)	0.016

Organ dysfunction at the time of mNGS testing, n (%)										
Respiratory	773 (85.0)	29 (74.4)	5 (55.6)	24 (80.0)	0.268	25 (78.1)	25 (78.1)	3 (50.0)	22 (84.6)	<0.001
Coagulation	301 (33.1)	15 (38.5)	4 (44.4)	11 (36.7)	0.112	14 (43.8)	12 (37.5)	3 (50.0)	9 (34.6)	0.128
Hepatic	138 (15.2)	3 (7.7)	1 (11.1)	2 (6.7)	0.237	1 (3.1)	3 (9.4)	1 (16.7)	2 (7.7)	0.260
Cardiovascular	476 (52.4)	14 (35.9)	1 (11.1)	13 (43.3)	0.336	14 (43.8)	13 (40.6)	1 (16.7)	12 (46.2)	0.063
Neurologic	351 (38.6)	14 (35.9)	4 (44.4)	10 (33.3)	0.056	13 (40.6)	13 (40.6)	3 (50.0)	10 (38.5)	<0.001
Kidney	201 (22.1)	9 (23.1)	3 (33.3)	6 (20.0)	0.023	7 (21.9)	9 (28.1)	3 (50.0)	6 (23.1)	0.145
Reads, median (IQR)	/	19 (5, 81.5)	380 (310, 2597)	10 (3.2, 33.2)		/	45.5 (9.8, 807.2)	1680.5 (454.2, 5392.2)	31 (9.2, 208.8)	
ICU admission to mNGS testing, days, median (IQR)	3 (2, 6)	3 (2, 6)	2(2, 3)	3.5(3, 6.8)	0.141	4 (2, 7)	3 (2, 6.2)	2 (2, 2.8)	3.5 (3, 7)	0.019
Anti-herpesvirus, n (%)										
Ganciclovir	20 (2.2)	3 (7.7)	1 (11.1)	2 (6.7)		1 (3.1)	2 (6.2)	0 (0.0)	2 (7.7)	
Acyclovir	9 (1.0)	2 (5.1)	0 (0.0)	2 (6.7)		1 (3.1)		2 (6.2)	0 (0.0)	2 (7.7)
Others§	2 (0.2)	1 (2.6)	0 (0.0)	1 (3.3)		0 (0.0)	1 (3.1)	0 (0.0)	1 (3.8)	

Notes: Propensity score matching was performed based on variables with the SMD more than 0.1.

SMD represented the difference between the herpesvirus-negative group and the herpesvirus-positive group.

#Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

§ Other antiviral drugs including penciclovir, famciclovir, etc.

Abbreviation: SMD, standardized mean difference; SD, standard deviation; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Figure S10

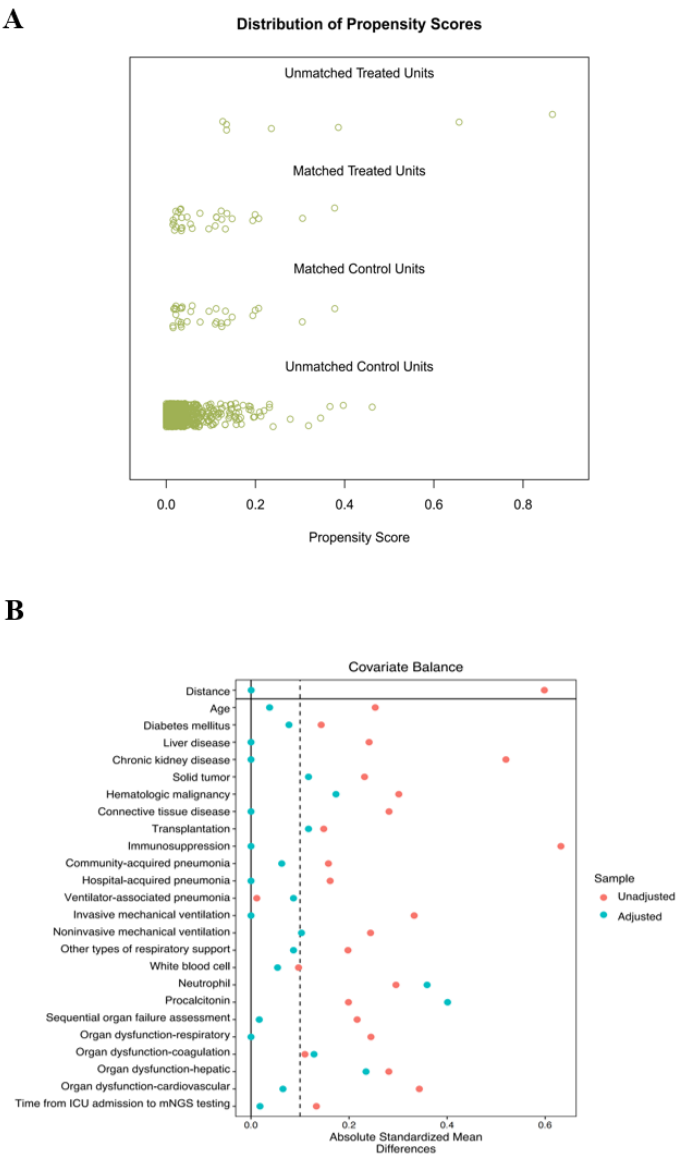


Figure legend:

Figure S10. Covariate pre and post balance plot of HHV-6B.

Figure S11

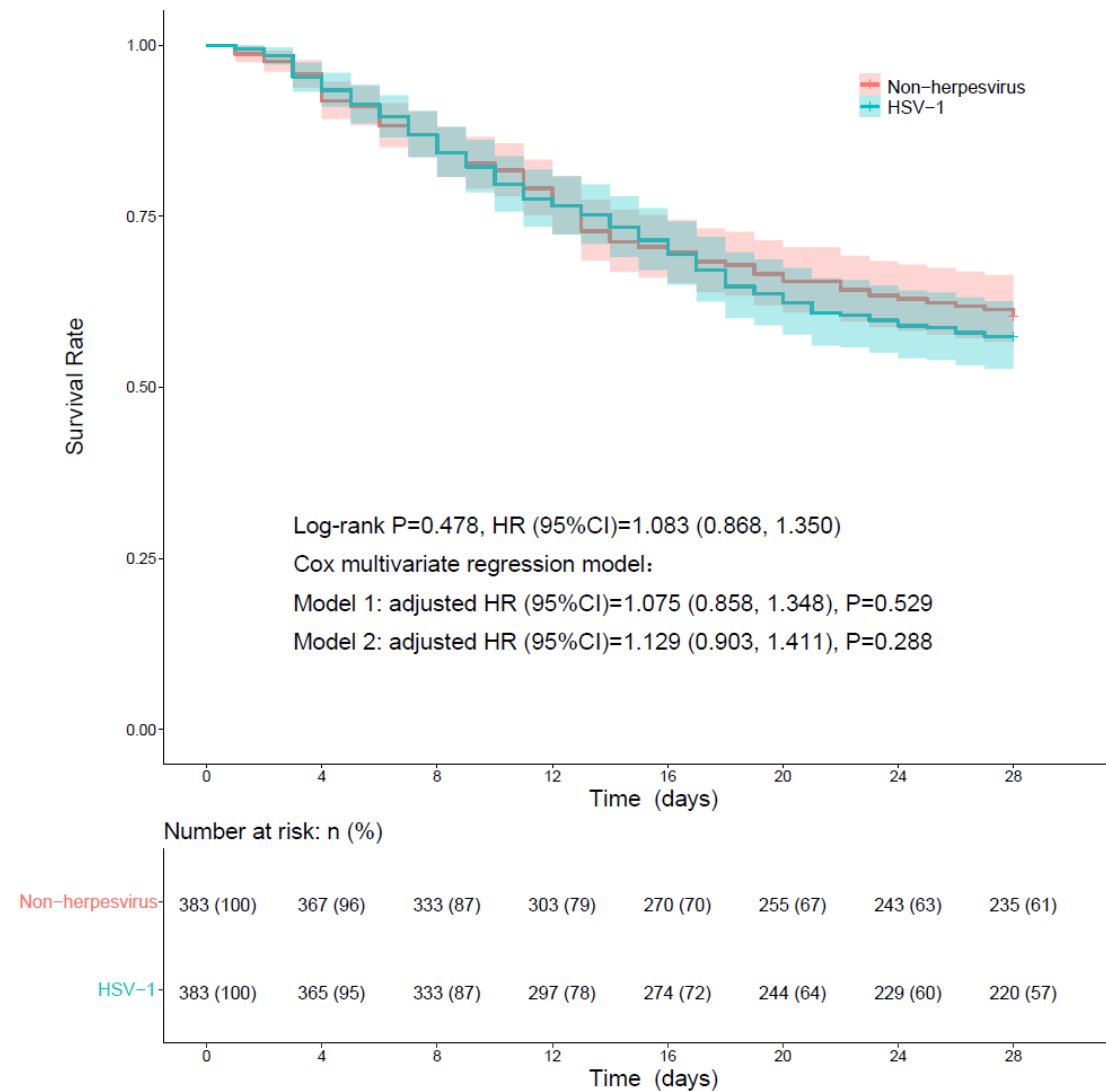


Figure legend:

Figure S11. Survival curve analysis of cohort after matching between HSV-1 positive and herpesvirus negative group.

Select the parameters based on historical confounders for multivariable Cox analysis. Model 1 includes other co-detected microorganisms with a P-value less than 0.1 in univariate Cox regression, while Model 2 does not include other co-detected microorganisms.

Figure S12

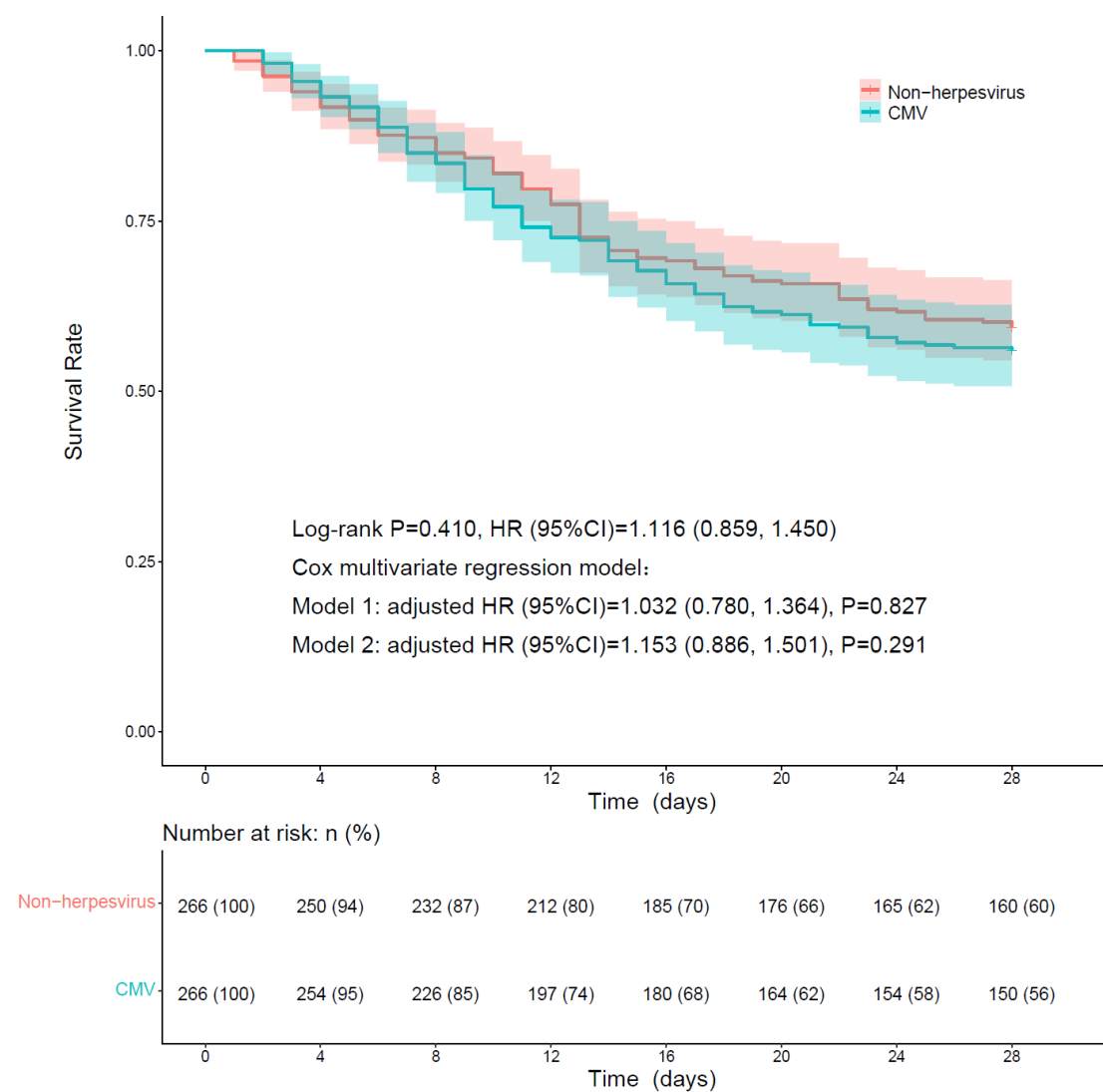


Figure legend:

Figure S12. Survival curve analysis of cohort after matching between CMV positive and herpesvirus negative group.

Selected parameters are based on historical confounders for multivariable Cox analysis. Model 1 includes other co-detected microorganisms with a P-value less than 0.1 in univariate Cox regression, while Model 2 does not include other co-detected microorganisms.

Figure S13

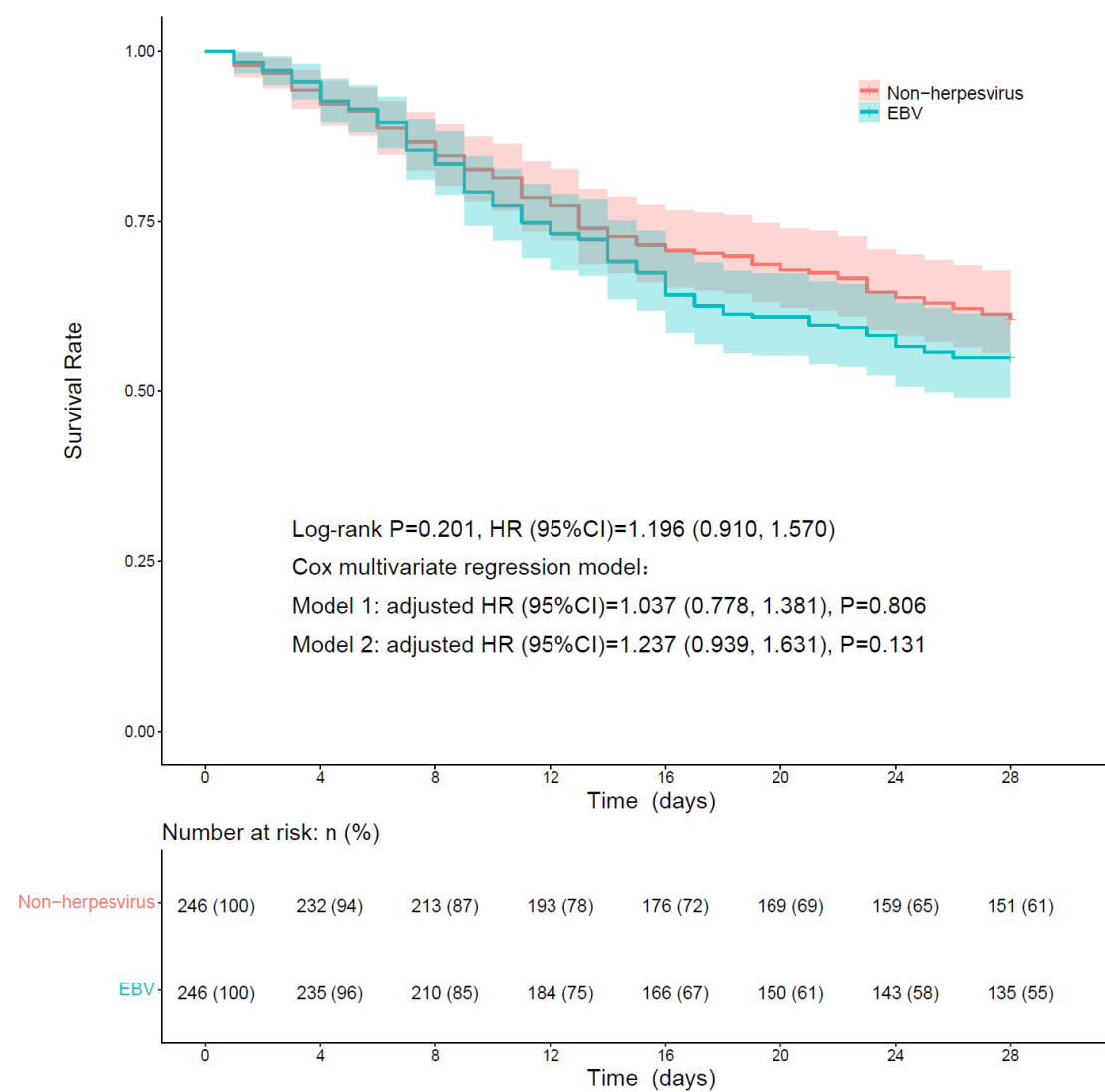


Figure legend:

Figure S13. Survival curve analysis of cohort after matching between EBV positive and herpesvirus negative group.

Selected parameters are based on historical confounders for multivariable Cox analysis. Model 1 includes other co-detected microorganisms with a P-value less than 0.1 in univariate Cox regression, while Model 2 does not include another co-detected microorganism.

Figure S14

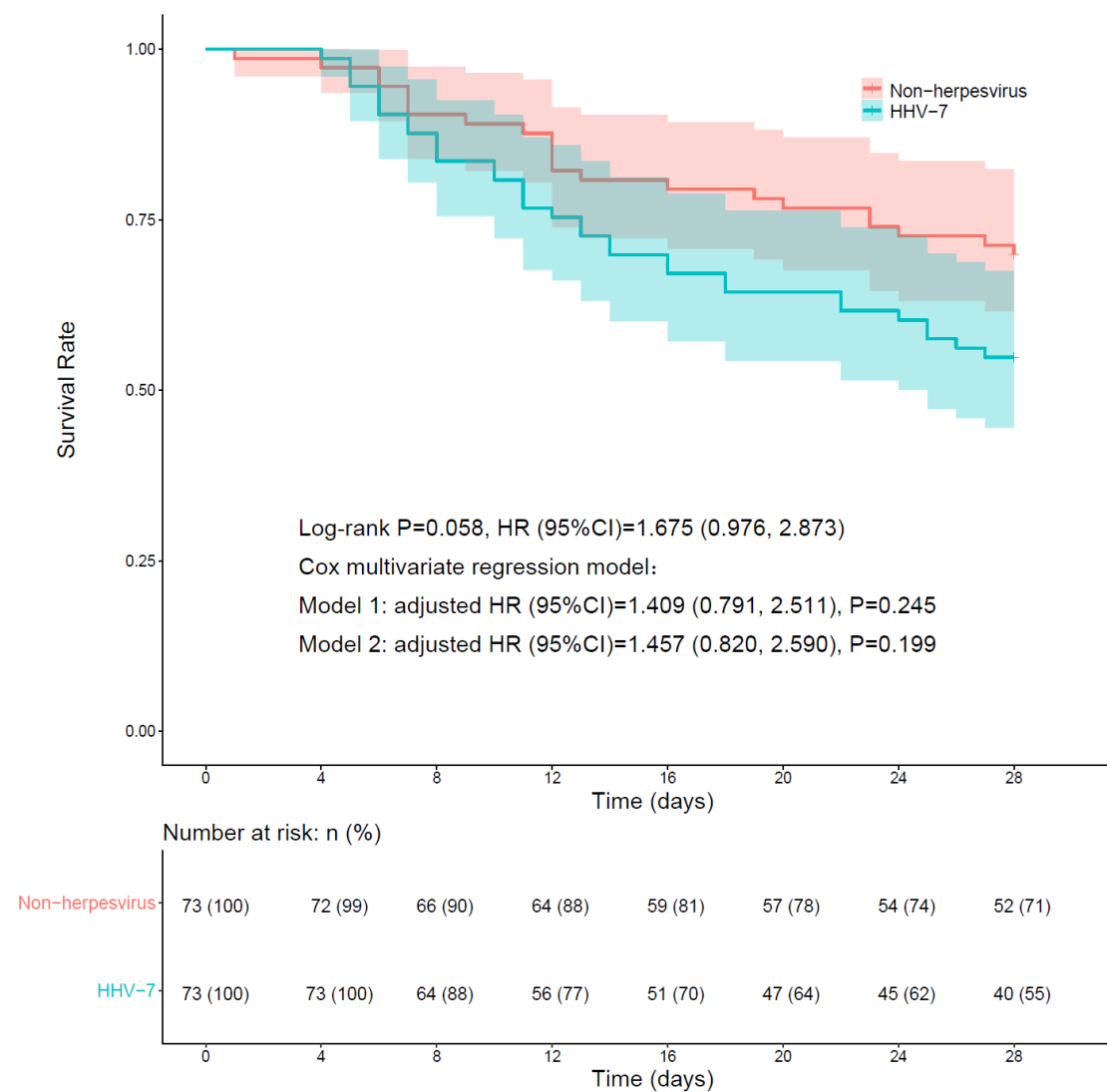


Figure legend:

Figure S14. Survival curve analysis of cohort after matching between HHV-7 positive and herpesvirus negative group.

Selected parameters are based on known confounders for multivariable Cox analysis. Model 1 includes other co-detected microorganisms with a P-value less than 0.1 in univariate Cox regression, while Model 2 does not include other co-detected microorganisms.

Figure S15

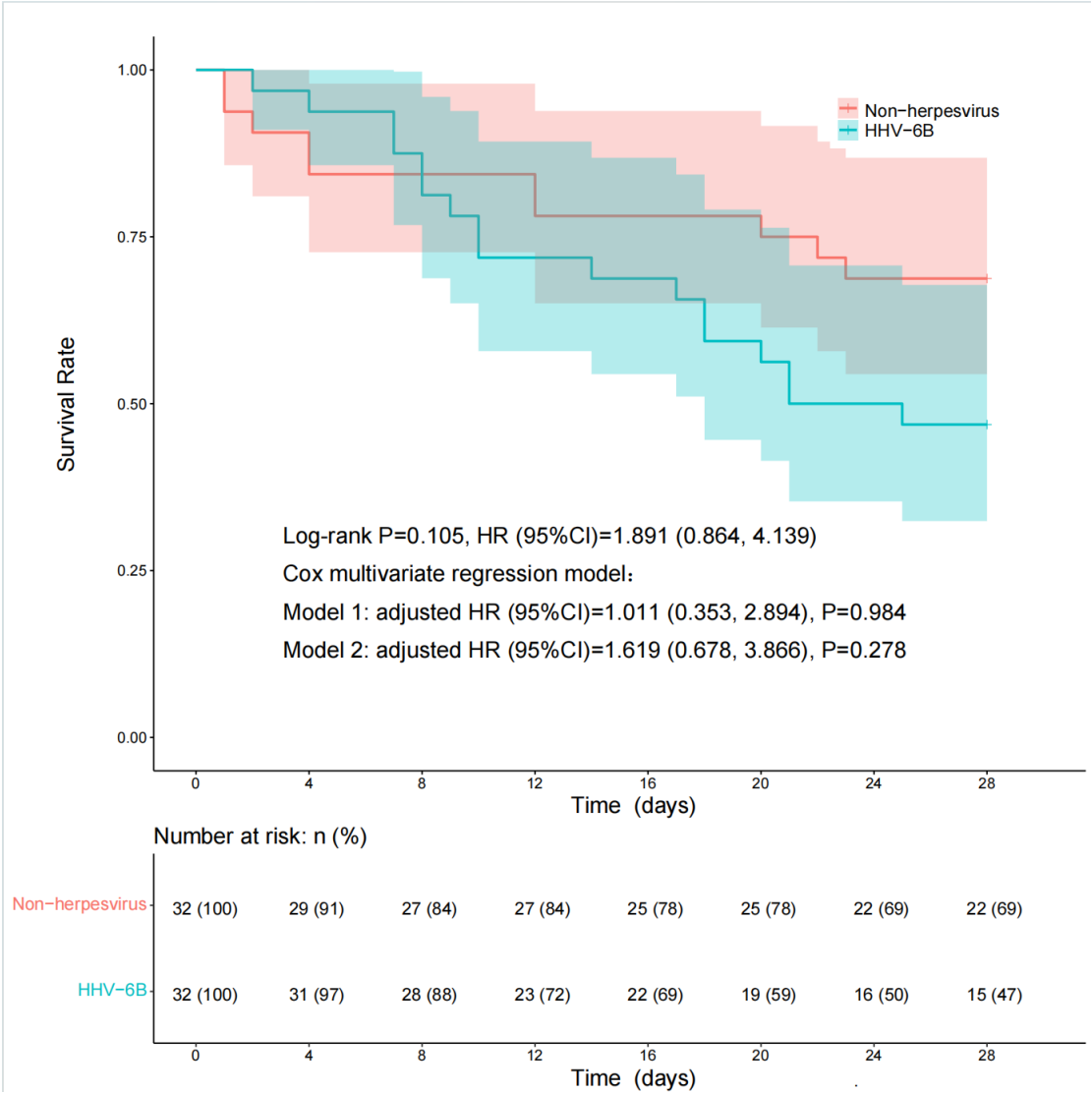


Figure legend:

Figure S15. Survival curve analysis of cohort after matching between HHV-6B positive and herpesvirus negative group.

Selected parameters are based on historical confounders for multivariable Cox analysis. Model 1 includes other co-detected microorganisms with a P-value less than 0.1 in univariate Cox regression, while Model 2 does not include other co-detected microorganisms.

Figure S16

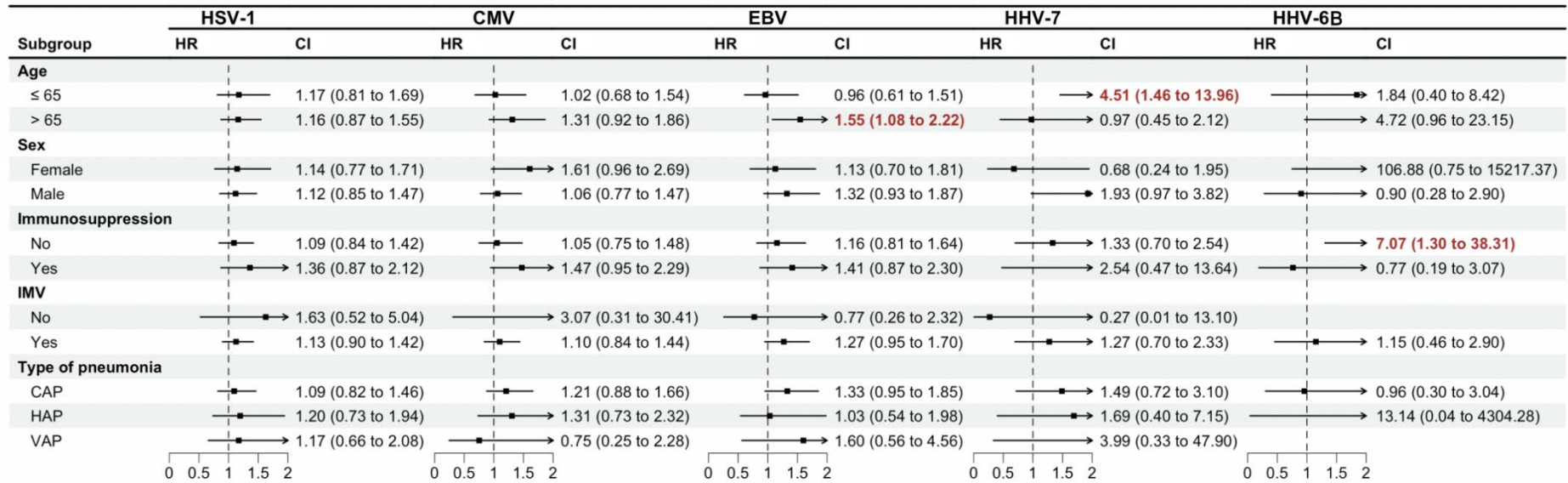


Figure legend:

Figure S16. Subgroup analysis of 28-day mortality in different herpesviruses positive group.

Bold red font indicates statistical differences (P < 0.05).

Figure S17

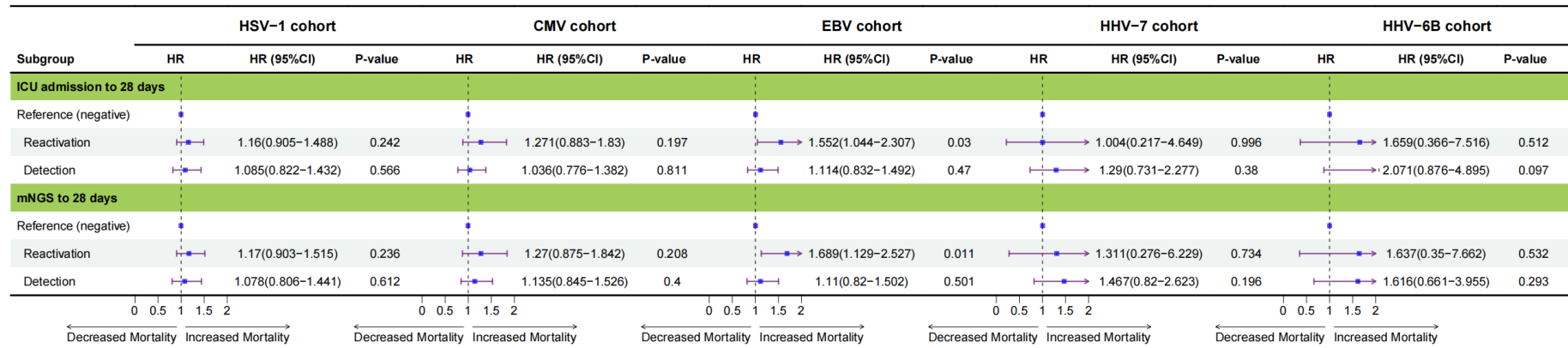


Figure legend:

Figure S17. Cox model analysis of herpesvirus reactivation and detection on 28-day mortality after metagenomic testing and ICU admission in matched cohorts. Select parameters HR for multivariable Cox analysis based on historical confounders.

Figure S18

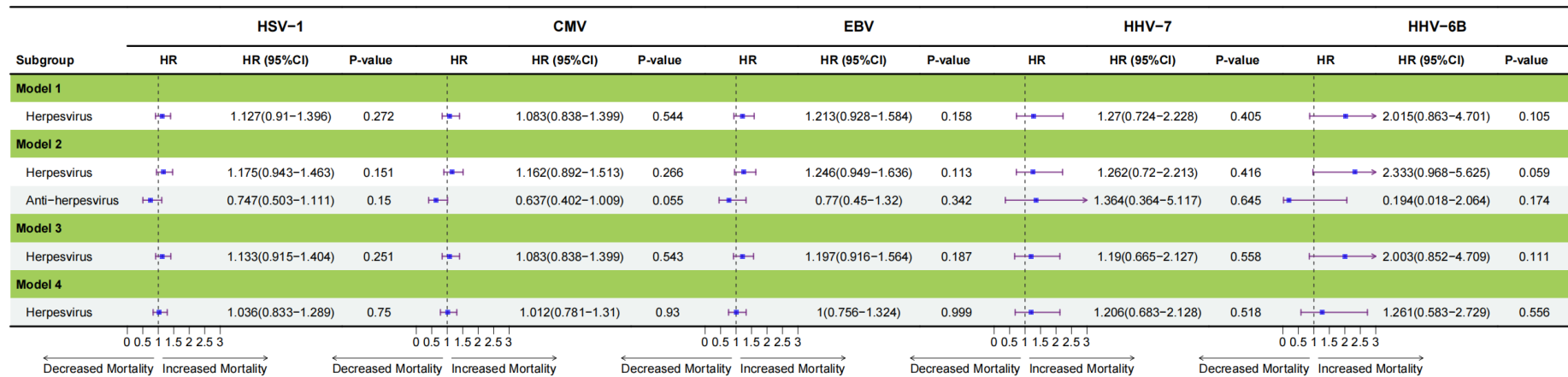


Figure legend:

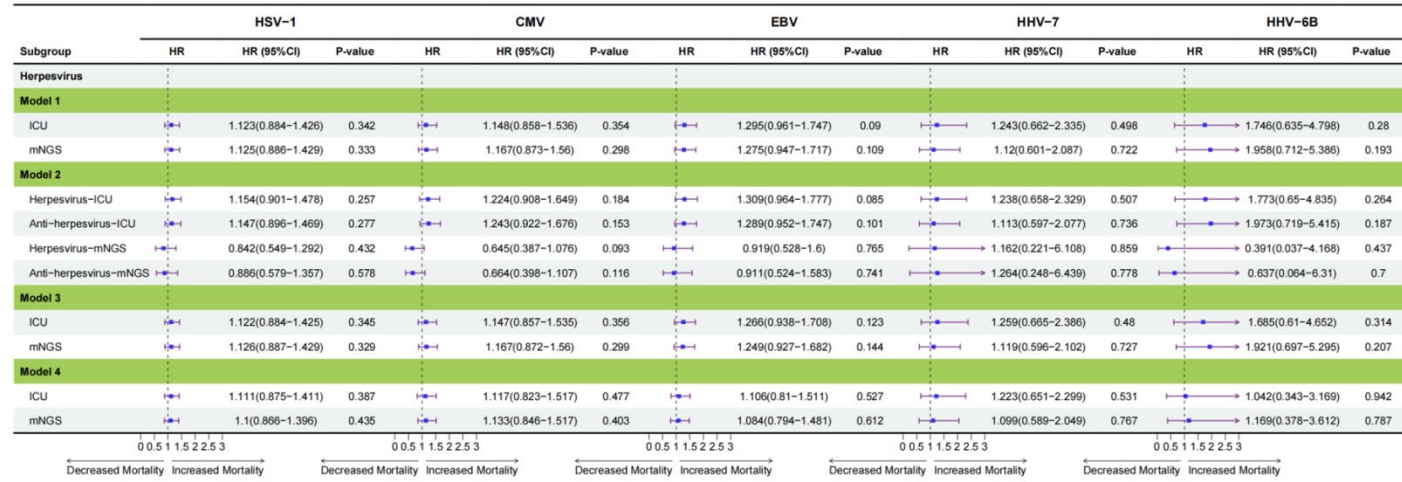
Figure S18. Sensitivity analysis was conducted on patients who died within 28 days after metagenomic testing.

Multivariable Cox regression analysis was used to analyze the association between different herpesvirus cohorts and the mortality risk.

Select parameters for multivariable Cox analysis based on historical confounders. Model 1 includes only historical confounders, while Models 2, 3, and 4 include these historical confounders as well as anti-herpesvirus treatment, corticosteroids, and other co-detected microorganisms with a P-value less than 0.1 in univariate Cox regression.

Figure S19

A



B

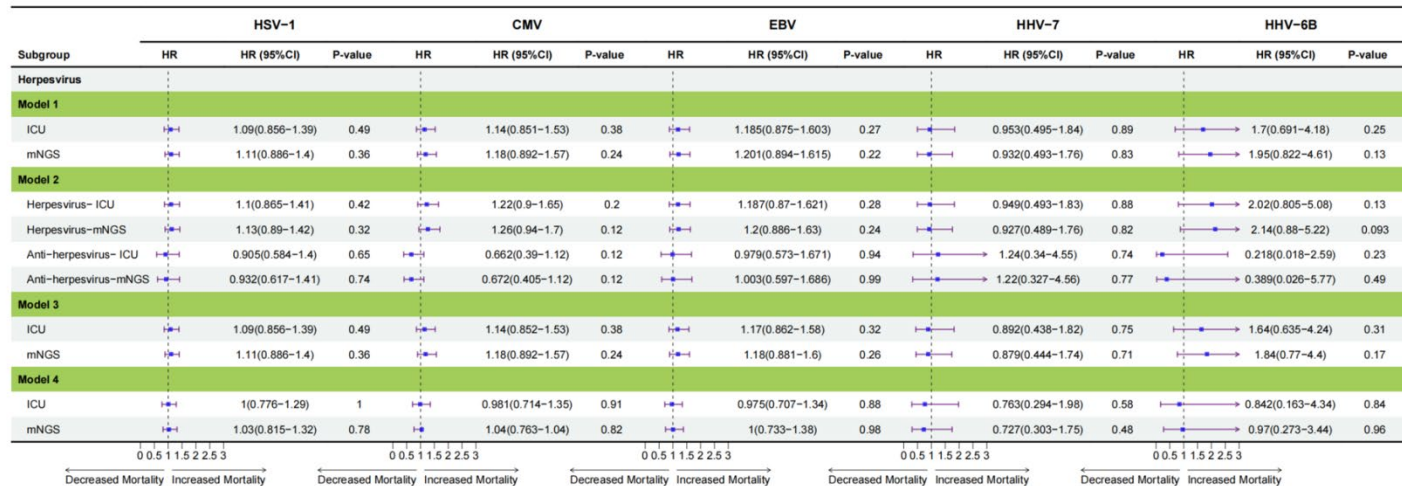


Figure legend:

Figure S19. Competing risks model analysis was conducted in the matched cohorts of different herpesviruses.

Extubation was considered a competing risk in the analysis for patients who died after intubation. These patients included those who died within 28 days after ICU admission and those who died within 28 days after metagenomic testing. A and B respectively illustrate the analysis using cause-specific hazard ratios and subdistribution hazard ratios, two types of competing risk models.

Select parameters for multivariable Cox analysis based on historical confounders. Model 1 includes only historical confounders, while Models 2, 3, and 4 include these historical confounders as well as anti-herpesvirus treatment, corticosteroids, and other co-detected microorganisms with a P-value less than 0.1 in univariate Cox regression.

Table S11. Comparison of clinical characteristics of patients with different HSV-1 and CMV statuses

Variables	Both positive (n=114)	HSV-1 positive (n=352)	CMV positive (n=195)	Both negative (n=1076)	P value
Age, years, median (IQR)	68 (62.2, 76)	67 (55, 75)	65 (54, 74)	67 (55, 75)	0.062
Male gender, n (%)	79 (69.3)	252 (71.6)	144 (73.8)	741 (68.9)	0.478
Comorbidities, n (%)					
Diabetes mellitus	28 (24.6)	80 (22.7)	45 (23.1)	274 (25.5)	0.719
Myocardial infarction	8 (7.0)	20 (5.7)	8 (4.1)	67 (6.2)	0.655
Chronic pulmonary disease	27 (23.7)	81 (23.0)	47 (24.1)	195 (18.1)	0.062
Liver disease	11 (9.6)	31 (8.8)	21 (10.8)	55 (5.1) ^c	0.004
Chronic kidney disease	25 (21.9)	38 (10.8) ^a	40 (20.5) ^b	116 (10.8) ^{ac}	<0.001
Solid tumor	13 (11.4)	56 (15.9)	40 (20.5)	152 (14.1)	0.083
Hematologic malignancy	8 (7.0)	10 (2.8)	18 (9.2) ^b	55 (5.1)	0.011
Connective tissue disease	5 (4.4)	9 (2.6)	18 (9.2) ^b	42 (3.9) ^c	0.002
Transplantation	8 (7.0)	11 (3.1)	23 (11.8) ^b	46 (4.3) ^c	<0.001
Cerebrovascular disease	22 (19.3)	46 (13.1)	26 (13.3)	185 (17.2)	0.150
Immunosuppression, n (%)	45 (39.5)	74 (21.0) ^a	83 (42.6) ^b	228 (21.2) ^{ac}	<0.001
Type of pneumonia, n (%)					<0.001
CAP	70 (61.4)	195 (55.4)	121 (62.1) ^b	665 (61.8) ^b	
HAP	26 (22.8)	78 (22.2)	59 (30.3) ^b	256 (23.8) ^b	
VAP	18 (15.8)	79 (22.4)	15 (7.7) ^b	155 (14.4) ^b	
Respiratory support, n (%)					0.733
IMV	97 (85.1)	301 (85.5)	170 (87.2)	942 (87.5)	
NIV	4 (3.5)	8 (2.3)	7 (3.6)	33 (3.1)	
Others [#]	13 (11.4)	43 (12.2)	18 (9.2)	101 (9.4)	
Duration of IMV, median (IQR)	9 (3, 17)	9 (3, 17)	8 (4, 15)	8 (3, 15)	0.794
Ventilator-free days at day 28, median (IQR)	0 (0, 7)	2 (0, 16) ^a	1 (0, 17) ^a	1 (0, 18) ^a	0.016
Laboratory tests, median (IQR)					
White blood cell ($\times 10^9/L$)	10.9 (7.2, 15.3)	11.8 (7.3, 16.9)	10.7 (6.9, 15.6)	11.0 (6.8, 15.6)	0.356
Lymphocyte ($\times 10^9/L$)	0.4 (0.2, 0.7)	0.6 (0.3, 1.1) ^a	0.5 (0.3, 0.9)	0.6 (0.3, 1.0) ^{ac}	<0.001
Neutrophil ($\times 10^9/L$)	9.4 (6.1, 14.6)	9.9 (5.9, 14.7)	9.3 (5.8, 13.6)	9.4 (5.5, 13.9)	0.476
C-reactive protein (mg/L)	72.7 (29.0, 147.3)	83.0 (26.1, 158.6)	91.7 (43.7, 148.4)	90.5 (30.0, 163.6)	0.551
Procalcitonin (ng/ml)	0.9 (0.3, 3.1)	0.8 (0.2, 3.5)	0.9 (0.3, 4.9)	0.9 (0.2, 6.8)	0.911
SOFA, median (IQR)	8 (6, 10.8)	7 (5, 10)	7 (5, 10)	7 (5, 10)	0.283

Organ dysfunction, n (%)					
Respiratory	97 (85.1)	287 (81.5)	170 (87.2)	912 (84.8)	0.323
Coagulation	46 (40.4)	139 (39.5)	77 (39.5)	367 (34.1)	0.142
Hepatic	16 (14.0)	67 (19.0)	32 (16.4)	162 (15.1)	0.323
Cardiovascular	62 (54.4)	161 (45.7)	99 (50.8)	545 (50.7)	0.301
Neurologic	42 (36.8)	133 (37.8)	54 (27.7)	407 (37.8)	0.056
Kidney	33 (28.9)	64 (18.2)	53 (27.2)	241 (22.4)	0.030
Hospital stays, median (IQR)	22 (11, 39.8)	24 (14, 37)	21 (12, 41)	20 (12, 34) ^b	0.024
ICU stay, median (IQR)	15 (8, 24)	15 (8.8, 26)	12 (7, 21)	12 (7, 23) ^b	0.006
Time from ICU admission to mNGS testing, median (IQR)	4 (2, 9)	4 (2, 10)	3 (2, 5) ^{ab}	3 (2, 6) ^{ab}	<0.001
28-day mortality in ICU, n (%)	63 (55.3)	138 (39.2) ^a	81 (41.5)	436 (40.5) ^a	0.018

Notes:

To control for the effects of multiple testing, Bonferroni correction was applied for comparisons between groups. In the table, the symbols ^a, ^b, and ^c indicate statistical significance between groups, where these symbols represent an adjusted *P* value < 0.05.

^a Compared with Both positive group;

^b Compared with HSV-1 positive group;

^c Compared with CMV positive group;

[#] Other types of respiratory support including high-flow nasal cannula, Venturi mask, etc.

Abbreviations: IQR, interquartile range; CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; IMV, invasive mechanical ventilation; NIMV, noninvasive mechanical ventilation; SOFA, sequential organ failure assessment; ICU, intensive care unit; NGS, next generation sequencing.

Table S12. Comparison of microbial characteristics of patients with different HSV-1 and CMV statuses

Variables	Both positive (n=114)	HSV-1 positive (n=352)	CMV positive (n=195)	Both negative (n=1076)	<i>P</i> value
Herpesviridae, n (%)					
<i>Epstein–Barr virus</i>	50 (43.9)	59 (16.8) ^a	55 (28.2) ^{ab}	125 (11.6)^{ac}	<0.001
<i>Human herpesvirus-6B</i>	6 (5.3)	7 (2.0)	9 (4.6)	17 (1.6)	0.009
<i>Human herpesvirus-7</i>	10 (8.8)	20 (5.7)	22 (11.3)	40 (3.7) ^c	<0.001
<i>Varicella zoster virus</i>	2 (1.8)	0 (0.0)	4 (2.1)	2 (0.2) ^c	0.001
Culture, n (%)					
<i>Acinetobacter baumannii</i>	19 (16.7)	53 (15.1)	19 (9.7)	158 (14.7)	0.250
<i>Klebsiella pneumoniae</i>	7 (6.1)	41 (11.6)	15 (7.7)	120 (11.2)	0.179
<i>Pseudomonas aeruginosa</i>	7 (6.1)	17 (4.8)	18 (9.2)	66 (6.1)	0.239
<i>Stenotrophomonas maltophilia</i>	10 (8.8)	22 (6.2)	10 (5.1)	39 (3.6)	0.029
<i>Burkholderia cepacia</i>	6 (5.3)	14 (4.0)	3 (1.5)	30 (2.8)	0.198
<i>Staphylococcus aureus</i>	2 (1.8)	5 (1.4)	3 (1.5)	26 (2.4)	0.727
<i>Escherichia coli</i>	1 (0.9)	7 (2.0)	3 (1.5)	16 (1.5)	0.866
<i>Corynebacterium striatum</i>	3 (2.6)	13 (3.7)	5 (2.6)	15 (1.4)	0.046
Clinical metagenomics, n (%)					
<i>Acinetobacter spp.</i>	43 (37.7)	149 (42.3)	44 (22.6) ^{ab}	293 (27.2) ^b	<0.001
<i>Klebsiella spp.</i>	33 (28.9)	124 (35.2)	39 (20.0) ^b	302 (28.1)	0.002
<i>Pseudomonas spp.</i>	26 (22.8)	60 (17.0)	28 (14.4)	167 (15.5)	0.194
<i>Stenotrophomonas spp.</i>	25 (21.9)	75 (21.3)	31 (15.9)	143 (13.3) ^b	<0.001
<i>Enterococcus spp.</i>	34 (29.8)	77 (21.9)	32 (16.4)	156 (14.5)^{ab}	<0.001
<i>Burkholderia spp.</i>	16 (14.0)	57 (16.2)	13 (6.7) ^b	75 (7.0) ^b	<0.001
<i>Staphylococcus spp.</i>	14 (12.3)	30 (8.5)	9 (4.6)	113 (10.5)	0.045
<i>Corynebacterium spp.</i>	6 (5.3)	32 (9.1)	9 (4.6)	60 (5.6)	0.078
<i>Escherichia spp.</i>	4 (3.5)	23 (6.5)	8 (4.1)	48 (4.5)	0.359
<i>Streptococcus spp.</i>	5 (4.4)	16 (4.5)	10 (5.1)	120 (11.2) ^b	<0.001
<i>Haemophilus spp.</i>	7 (6.1)	17 (4.8)	3 (1.5)	56 (5.2)	0.145
<i>Elizabethkingia spp.</i>	5 (4.4)	11 (3.1)	5 (2.6)	37 (3.4)	0.842
<i>Achromobacter spp.</i>	6 (5.3)	12 (3.4)	8 (4.1)	22 (2.0)	0.087
<i>Enterobacter spp.</i>	3 (2.6)	6 (1.7)	7 (3.6)	28 (2.6)	0.558
<i>Candida spp.</i>	37 (32.5)	122 (34.7)	53 (27.2)	320 (29.7)	0.225
<i>Pneumocystis spp.</i>	33 (28.9)	12 (3.4) ^a	58 (29.7) ^b	57 (5.3)^{ac}	<0.001
<i>Aspergillus spp.</i>	33 (28.9)	58 (16.5) ^a	31 (15.9)	152 (14.1)^a	<0.001
<i>Torque teno virus</i>	27 (23.7)	25 (7.1) ^a	32 (16.4) ^b	80 (7.4)^{ac}	<0.001
<i>Nakaseomyces spp.</i>	6 (5.3)	21 (6.0)	9 (4.6)	59 (5.5)	0.929
<i>Serratia spp.</i>	2 (1.8)	10 (2.8)	2 (1.0)	32 (3.0)	0.417

Notes: Only the 8 most frequently detected bacteria in sputum or respiratory aspirated cultures were displayed in the culture results.

To control for the effects of multiple testing, Bonferroni correction was applied for comparisons between groups. In the table, the symbols ^a, ^b, and ^c indicate statistical significance between groups, where these symbols represent an adjusted *P* value < 0.05.

^a Compared with Both positive group;

^b Compared with HSV-1 positive group;

^c Compared with CMV positive group;

Figure S20

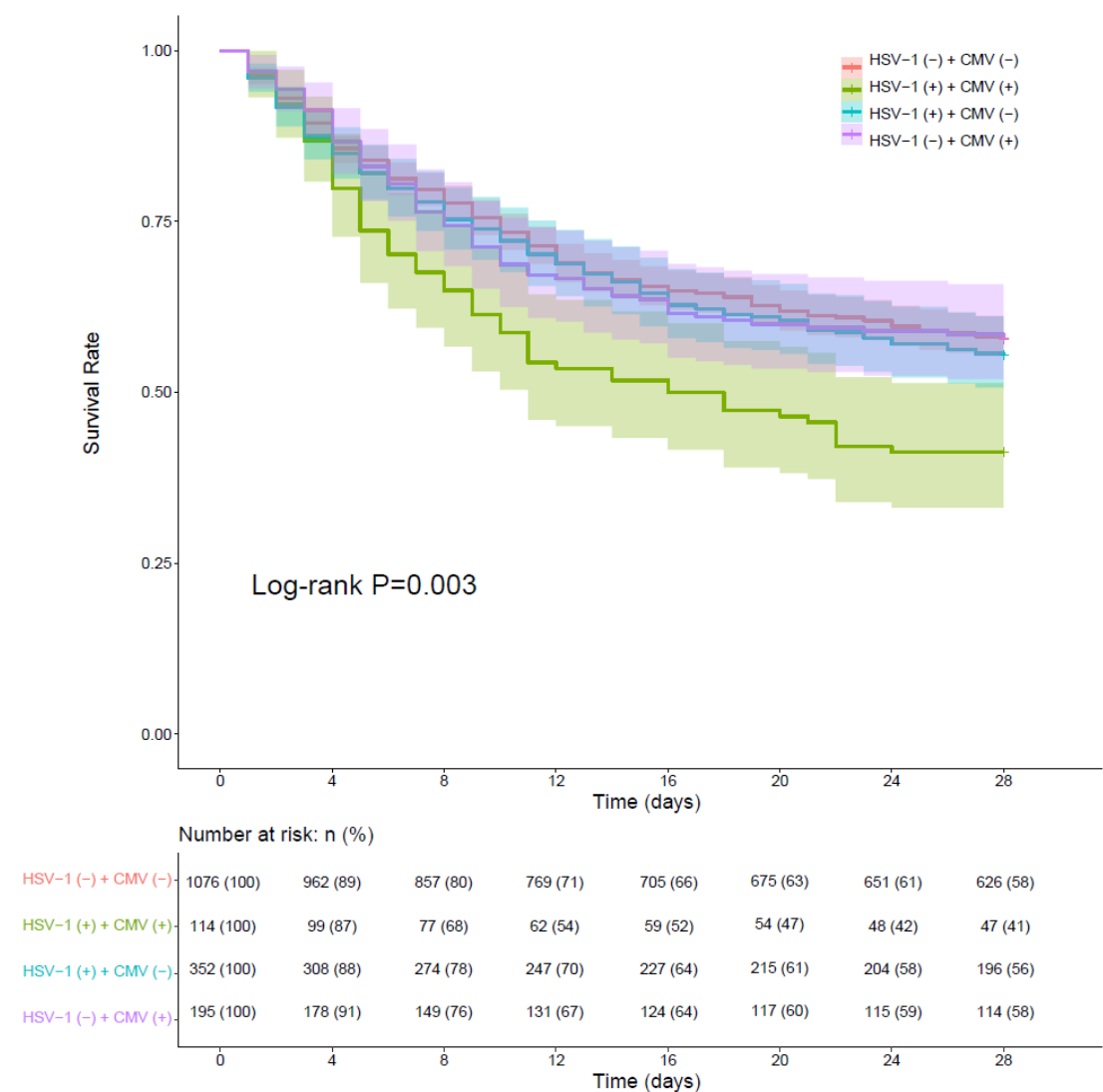


Figure legend:

Figure S20. Kaplan-Meier survival curves for 28-day survival of patients with different HSV-1 or CMV statuses after performing clinical metagenomics.

Kaplan-Meier survival analysis was used to analyze the association between different CMV and HSV positivity statuses after clinical metagenomic testing.